Improving the Success of Insect Conservation Translocations: A Case Study of the Nationally Endangered Robust Grasshopper (*Brachaspis robustus* Bigelow)

Jennifer Christina Schori

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The robust grasshopper *Brachaspis robustus*.
Photo: J. C. Schori

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Abstract

The current rates of species loss and decline are so extraordinary that the Earth is speculated to be on the cusp of entering a sixth mass extinction, with the majority of species lost expected to be insects. Insects make up approximately 70 % of all species on Earth and are proportionally the most under-represented class of animal in conservation biology. An important tool for substantially reducing the risk of extinction for critically threatened species is conservation translocation, which is defined by the International Union for Conservation of Nature as "the intentional movement and release of a living organism where the primary objective is a conservation benefit". However, there has been limited applications of translocation as a conservation tool for insects, and only 52 % of terrestrial insect translocations are reported as successful at establishing a persistent population. This thesis develops applied conservation management strategies to improve translocation success for insects using New Zealand's Nationally Endangered robust grasshopper, *Brachaspis robustus* Bigelow (Orthoptera: Acrididae), as a case study.

Brachaspis robustus is a large bodied, flightless, highly visually cryptic and non-stridulating grasshopper. It is a braided river specialist endemic to the Mackenzie Basin, an inter-montane dryland region in the centre of New Zealand's South Island. Currently all wild populations of *B. robustus* that are monitored by the New Zealand Department of Conservation show trends of decline. Despite being one of New Zealand's most threatened grasshopper species, little research has been directed toward maximising conservation outcomes for *B. robustus*.

The first objective of this research was to understand the life history of *B. robustus*. Grasshoppers were tracked from egg to adulthood in captivity in the field and in the laboratory. The life cycle of *B. robustus* was observed to be ~27 months in the field. Females laid on average 1.3 egg pods in the wild, but up to 8 in the laboratory. Egg pods contained between 17 and 35 eggs, and the eggs go through an obligate diapause which is almost certainly broken by cold winter temperatures below 0 °C. Survivorship was low in the laboratory and in the field, despite no predation pressure from key predators including birds and mammals. Understanding the life history of *B. robustus* has facilitated the interpretation of trends detected during population monitoring, informed the development of captive rearing for release protocols, and provided an opportunity to simulate expected outcomes of future translocations.

The second research objective was to understand the habitat requirements of *B. robustus*. Using miniaturised radio transmitters, the movements of adult female grasshoppers occupying a linear gravel road were compared to those occupying a more natural open braided river habitat. Dense vegetation was found to be unfavourable habitat, indicating that management of vegetation will be

important for maintaining habitat quality. No difference in home-range size was found between the two sites indicating that the area of habitat required to support an adult female is likely to be > 300 m^2. This has applications for managing remaining habitat (e.g. area over which management of weed and mammalian predators should be implemented), creating artificial habitat, and selecting potential receiving habitats for conservation translocations.

The third research objective of the current study was to evaluate the threat introduced mammalian predators pose to the persistence of *B. robustus*. The outcome of an experimental translocation where individuals were released into predator reduced and non-predator reduced areas was monitored. In addition, long-term trends were analysed of three populations of another declining dryland grasshopper species, *Sigaus minutus*, that are present in areas where mammalian predators are controlled at different levels of intensity. It was concluded that mammalian predators are likely to pose a substantial threat to *B. robustus,* and that high intensity mammalian predator control across the full suite of predators should be prioritised to improve conservation management and translocation success.

The fourth research object was to develop effective monitoring techniques for *B. robustus*. First, an intensive removal sampling study conducted over a single active season (November to March) was used to rapidly quantify seasonal and demographic variation in visual detectability of *B. robustus*. Juvenile instars were found to dominate population composition in all months except December (adults = 69 %) and males represented > 50 % of monthly captures. Adult females were 2-3 times larger than adult males, and 79 % of those captured were found during the first search of an area, compared to only 52 % of adult males. The odds of detecting an individual were found to increase by 6 % per 1 mm of body length. Second, by conducting experimental monitoring for three consecutive seasons, both population density and population distribution monitoring protocols were developed for *B. robustus.* The recommended population density monitoring protocol used adult female counts as an index of population size to maximise visual detectability and ensure data is biologically meaningful. November and early December was found to be the most appropriate time to conduct monitoring, and > 20 transect replicates with > 4 survey replicates each were required to detect a significant change in adult female population size with power > 0.8. Occupancy modelling was investigated as a distribution monitoring protocol for *B. robustus* by estimating the probability of detection (p_g) in a natural open riverbed compared to a gravel road habitat. Detection of grasshopper presence was found to be high (p_g > 0.6) when using a 100 m x 1 m transect in both habitat types under optimal (no cloud) conditions in February, and a minimum of 3 visits per season was required to have confidence in trend detection. Implementing the population monitoring protocol presented here will be important for measuring the outcome of any future translocations of *B. robustus*.

This research has contributed knowledge that has substantially advanced the understanding of a Nationally Endangered insect. It has provided evidence-based conservation management recommendations that contribute toward the development of conservation translocation as a successful and valuable tool for preventing future insect extinctions.

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Chapter 1 Introduction

Sánchez-Bayo and Wyckhuys (2019) predicted that 40 % of insect species globally will be extinct in forthcoming decades. Although criticised as sensationalist (Cardoso et al. 2019, Komonen et al. 2019, Thomas et al. 2019, Wagner 2019), the research has drawn much needed attention to the current rates of insect decline across the world. Protected areas in Germany have seen a decline of > 75 % of flying insect biomass in < 30 years (Hallmann et al. 2017), a third (33 %) of wild insect pollinators declined during a 23-year study in Great Britain (Powney et al. 2019), and almost 43 % of local Ephemeroptera in Czech Republic are in decline or extinct (Zahrádková et al. 2009). In New Zealand, threatened species are known in most insect Orders (Stringer and Hitchmough 2012) and include a high proportion of endemic species. For example, 28 % of all known Orthoptera species (Trewick et al. 2012), an order with 93 % endemism (Manaaki Whenua - Landcare Research New Zealand Organism Register 2019), and 8 % of all known Carabidae species, an order that boasts 92 % endemism (McGuiness et al 2007), are in decline or threatened with extinction. The current rates of species loss and decline are so extraordinary that the Earth is speculated to be on the cusp of entering a sixth mass extinction (Barnosky et al. 2011, Ceballos et al. 2015), with a majority of those losses expected to be insects (Thomas et al. 2004, Régnier et al. 2015). Dunn (2005) conservatively estimated that 57,000 insect species per million species of eukaryotes could go extinct in the next 50 years which, when based on an estimate of 8.7 million species of eukaryotes on Earth (Mora et al. 2011), equates to nearly 10,000 insect species lost per year.

Insects make up approximately 70 % of all species on Earth (Samways 2018), and are the most diverse group of metazoans on the planet (Finlay et al. 2006). There are between 720,000 (May 2000) and ~1 million (Chapman 2009) described species, and a further 2 million (Nielsen and Mound 2000) to 8 million (Hammond 1995, Groombridge and Jenkins 2002) species estimated to be undescribed. In New Zealand there is estimated to be ~20,000 species of insects (McGuinness 2001) of which just over half have been formally described (Cranston 2010), and 80 % to 90 % are expected to be endemic (McGuinness 2001, Department of Conservation 2017). Despite representing a huge proportion of the planet's biodiversity, insects are not prioritised in conservation biology (Clark and May 2002, New and Samways 2014, Donaldson et al. 2016). Proportionally, insects are the most under-represented class of animal in conservation biology, which is biased towards large charismatic terrestrial vertebrates and birds in particular (Troudet et al. 2017). In the European Union, fewer than 7 % of the 800 conservation projects funded by the LIFE Programme were dedicated to invertebrates (Mammides 2019). In New Zealand, the Department of Conservation Threatened Species Strategy (Department of

Conservation 2017) includes only 16 insect species (1 % of the 1,247 invertebrate species currently ranked Threatened or At Risk (Stringer and Hitchmough 2012)) in its list of 150 priority species compared to 39 bird species (22 % of the 178 species currently ranked Threatened or At Risk (Robertson et al. 2017)), despite estimates that conservation of insects costs almost a third less financially per species than birds (Gordon et al. 2019).

Two general approaches to conservation that can be applied to insects are conservation at the land-scape scale, and conservation at the species scale (Samways 2018). Approaching conservation at the landscape scale is advantageous because the benefits reach many species and conserve their interactions within the ecosystem (Franklin 1993). Taking a species scale approach can appear restricted in contrast because initially the benefits are constrained to a single species. However, a single species approach can lead to the development of novel conservation techniques that can be adapted for application to other species (Samways 2018). When it involves habitat protection, the benefits can extend to other species also living in that area (Caro and O'Doherty 1999). Some species may also require immediate and targeted conservation effort because they face immediate threat of extinction. In these cases, a species-specific conservation approach is essential, particularly when a landscape scale approach is slow to implement or take effect.

1.1. Translocation as a conservation tool for insects

A conservation translocation is defined by the International Union for Conservation of Nature (IUCN) as “the intentional movement and release of a living organism where the primary objective is a conservation benefit” (IUCN 2013). It can be an important tool for substantially reducing the risk of extinction for a species (Sherley 1998, Seddon et al. 2014). The IUCN broadly recognises four types of conservation translocations; 1) reinforcement, where individuals are released within a population of conspecifics for the purpose of increasing, for example, population size, or genetic diversity; 2) reintroduction, where individuals are released into a new location within their range; 3) assisted colonisation, where individuals are released to an area outside of their range to establish a new population; and 4) ecological replacement, where individuals are released outside of their range to fulfil a specific ecological role (IUCN 2013). Translocations can be used to release a species from specific drivers of population decline, or to increase the geographic distribution of the species to safeguard against stochastic events or predicted future threats such as climate change (IUCN 2013). Henceforth, use of the word ‘translocation’ in this study will refer specifically to assisted colonisation and reintroduction.

There has been limited application of translocation as a conservation strategy for insects. Globally, between 3 % (Bajomi et al. 2010) and 7 % of conservation translocations have been conducted for terrestrial invertebrates, compared to > 95 % for birds and mammals (Fischer and Lindenmayer 2000). In New Zealand, only 15 insect species (1 % of 1,247 At Risk or Threatened invertebrate taxa (Stringer and Hitchmough 2012)) have undergone a translocation (Sherley et al. 2010), and only 5 % of translocation proposals approved in the 8 years between 2002 and 2010 were for invertebrates (Cromarty and Alderson 2013). This is despite the fact that many insects face similar threats as other threatened fauna in New Zealand (McGuinness 2001, Lester et al. 2014) and are likely to benefit from a translocation to locations where those threats are removed or substantially mitigated, and that insects generally lend themselves well to translocation (Pearce-Kelly et al. 1998, Hochkirch et al. 2007). Compared to mammals and birds, insects have a small body size making the logistics of collecting, housing and moving individuals from one place to another simpler in many cases (Pearce-Kelly et al. 1998, Hochkirch et al. 2007). Furthermore, high rates of fecundity mean that once released within a suitable and favourable receiving habitat, population growth can occur relatively quickly if threats causing decline in the source habitat have been removed (Pearce-Kelly et al. 1998, Hochkirch et al. 2007).

1.2. Improving translocation success

A translocation is often considered to be successful when a self-sustaining population that requires no further human intervention establishes and persists for several generations within the receiving habitat. Only 52 % of terrestrial insect translocations globally have been successful at establishing a population that persists for at least one generation (Bellis et al. 2019). Almost a third (31 %) of terrestrial insect translocations have failed and the remaining 17 % have undetermined outcomes because of a lack of post-release monitoring (Bellis et al. 2019). Translocations of threatened species can be susceptible to failure in part because founder populations contain few individuals making them vulnerable to stochastic environmental and demographic events that can occur before a viable population has established (Berggren 2001). Beyond increasing the number of individuals that constitute the founder population, it can be challenging to combat the effects of stochastic events in small populations (Knafler et al. 2017). Several other considerations that can improve translocation success include providing appropriate habitat, adequate nutrition, suitable mates and minimal predation pressure (Armstrong et al. 2015).

Understanding the species biology can greatly improve translocation success. Forecasting translocation outcomes by modelling or simulating population growth often requires users to input

life history data such as estimated reproductive output or survival rates (Armstrong and Seddon 2008, Weiser et al. 2013). Post-release survival can also be strongly influenced by the timing of release in relation to the species biological cycles (Armstrong et al. 2015). For example, individuals that are likely to disperse for mate finding (Chapman and Joern 1990, Berggren 2001) will be poor candidates for translocation as they are biologically predisposed to exhibit dispersal behaviours that could reduce the size of the founder population. An understanding of the requirements for growth, reproduction and courtship can improve selection of receiving habitats, particularly for insects, where the habitat requirements for egg development are quite different compared to those of adults or nymphs (Wünsch et al. 2012).

Another critical consideration of a translocation is selecting an appropriate receiving habitat. Given the goal of a translocation is to establish a population, it is important that the size of the receiving habitat and the resources available within it are sufficient for population growth. For insects, population growth can occur relatively quickly. For example, a translocated population of the endangered field cricket *Gryllus campestris* increased by 1,140 % in 3 years, rapidly expanding the occupied area from 5.66 ha to 33.14 ha (Hochkirch et al. 2007). Another important consideration is that the driver of population decline is absent, or substantially reduced, within the receiving habitat (IUCN 2013). In New Zealand, a number of insect translocations moved species onto mammal-free off-shore islands to release them from the pressure of introduced mammalian predators present on the mainland (Watts et al. 2008). However, if the threat cannot be eliminated, then it is important that it is substantially reduced, or that the receiving habitat provides adequate refuges for translocated individuals to avoid the threat.

Dispersal away from a translocation site is common but is detrimental to translocation success because it results in the loss of individuals from an already small founder population. Selecting high quality, favourable habitat can substantially reduce dispersal away from the translocation site because individuals do not leave in search of more optimal habitat (Armstrong and Seddon 2008). For example, dispersal rates of grasshoppers can generally be predicted by food quality and quantity, along with the availability of basking and oviposition sites (Chapman and Joern 1990). Changes to movement patterns can also occur in response to an unfamiliar environment (Armstrong and Seddon 2008). The grasshopper *Oedipoda caerulescens* moved longer distances with smaller turning angles (travel was more directional) when released in an unfamiliar environment compared to individuals released within a familiar environment, an effect that was most distinct on the first day of release (Heidinger et al. 2009). Implementing a 'soft-release' approach, where individuals are not completely emancipated until after an adjustment period, can reduce dispersal upon release because it gives animals an opportunity to become familiar with the new habitat (Armstrong and McLean 1995).

Having an appropriate monitoring regime to measure progress of the translocation is vital for achieving conservation success. Approximately 50 % of translocations have unknown outcomes because effective monitoring techniques were not implemented (Fischer and Lindenmayer 2000). Lack of monitoring following the translocation confounds the identification of causes of failure and impedes advancement of translocation design and protocols (Seddon et al. 2014). For example, two New Zealand wētā species, the Cook Strait giant wētā (*Deinacrida rugosa)* and Mahoenui giant wētā (*Deinacrida mahoenui*) have undergone multiple conservation translocations since 1976, driven by threats to habitat and predation by introduced mammals (Meads 1995). However, early translocations did not implement post-release monitoring, so it is not known whether they were successful or not (Watts et al. 2008, Watts and Thornburrow 2009). Modern translocations of wētā are now monitored using non-invasive wētā motels and harmonic radio transponders, which have allowed release site habitat to be critically evaluated, and factors contributing to translocation success or failure to be identified (Watts et al. 2012).

1.3. Conservation translocation of the robust grasshopper, *Brachaspis robustus*

The Nationally Endangered robust grasshopper, *Brachaspis robustus* Bigelow (Orthoptera: Acrididae) (Stringer and Hitchmough 2012) is a flightless short-horned grasshopper endemic to the inter-montane dryland region in the centre of New Zealand's South Island known as the Mackenzie Basin[1] (Trewick et al. 2012). The grasshopper is a braided river specialist that prefers open gravel habitat found on alluvial flood plains, braid islands and associated river terraces (White 1994). Braided rivers are characterised by highly variable flows and wide gravel flood plains. They are high disturbance, dynamic environments with regular flooding events that can often change channel morphology and braid dynamics (Gray and Harding 2007). The current range of *B. robustus* is restricted to just three river catchments in the region, Ōhau, Pūkaki and Tekapō.

A characteristically broad pronotum and large size give *B. robustus* its renowned 'robust' appearance (Bigelow 1967) and its common name, 'robust grasshopper'. It is thought that *B. robustus* is a generalist herbivore, feeding on mosses, lichens and leafy vegetation. Adult females, which can reach up to 38 mm in body length, are much larger than adult males which reach up to 17 mm in body length. Both sexes are highly visually cryptic, with pale to dark grey colouring tinged with greens, browns or black that mimic the stones and silts that are characteristic of its habitat. Neither sex is

[1]The Mackenzie Basin is also frequently referred to as the 'Upper Waitaki Basin' and occasionally as the 'Mackenzie Country' or the 'Pūkaki-Tekapō Basin'.

stridulating, meaning they also acoustically cryptic, however, no ultrasonic testing has been conducted.

Very little is currently known about the life cycle of *B. robustus.* The first and only description is based on evidence recorded during monthly visits to a single population over two summer periods (White 1994). Instars representing all stages of development were found throughout the year indicating a lack of synchronisation, and an observation of a juvenile overwintering suggested *B. robustus* has a semivoltine life cycle (White 1994). Although more is known about the life cycle of other grasshoppers in the endemic genus *Brachaspis* (Mason 1971), comparisons to *B. robustus* are limited because *B. collinus* and *B. nivalis* are alpine species occurring at 1,000 – 1,800 m above sea level (a.s.l.) (Hudson 1970), whereas *B. robustus* occurs below 800 m a.s.l.. Thermal extremes differ, and duration and timing of snow and subsequent melt occur at different times of the year for high altitude versus low altitude environments, meaning that the alpine grasshoppers will generally experience longer, colder winters than *B. robustus*.

Several recent changes to braided rivers and the surrounding land in the Mackenzie Basin region may have contributed to the current threatened status of *B. robustus*. The development of the Waitaki hydro scheme in the 1970s (McKay et al. 1978) has regulated river flow and reduced severity and occurrence of natural flooding events. Natural braid dynamics have also been altered by the encroachment of weedy species such as crack willow (*Salix fragilis*) and Russell lupin (*Lupinus polyphyllus*) (O'Donnell et al. 2016) that were historically planted to improve bank stability or provide shelter (Caruso 2006). Subsequently, the open exposed gravel which is the preferred habitat of *B. robustus* has become increasingly vegetated.

The other key potential threat to *B. robustus* is introduced mammalian predators. The fauna of New Zealand did not evolve with predatory land mammals. Consequently, many native and endemic species lack appropriate defence mechanisms for avoiding mammalian predation (Daugherty et al. 1993). As a result, the introduction of predatory mammals and their continued presence in native ecosystems has driven many endemic species to extinction, and many extant species are currently threatened or in decline (Holdaway 1989; Department of Conservation 2017). Because *B. robustus* is a large, flightless and ground-dwelling insect that relies on visual crypsis when threatened (Bigelow 1967; Morris 2002; Trewick et al. 2014), it is likely to be susceptible to predation by mammals. Visual crypsis does little to prevent detection by predatory mammals, which are often nocturnal, olfactory hunters (Gibbs 1998; Jones et al. 2005; Lester et al. 2014). Large insects are particularly vulnerable because they are often preferentially targeted by predatory mammals as a higher value food resource than smaller prey (St Clair 2011; Barker 2016). Previous research also shows diets of predatory

mammals in the Mackenzie Basin contain high proportions of invertebrates (Murphy et al. 2004; Jones et al. 2005; Dowding et al. 2015).

Currently all wild populations of *B. robustus* that are monitored by the Department of Conservation show trends of decline (Te Manahuna/Twizel DOC, pers. comms.). Population trends for *B. robustus* are generated from data collected using a twin transect walked by two observers on a single day in February (Te Manahuna/Twizel DOC, pers. comms.). The method relies on visual detection of individuals which usually depends upon the grasshopper jumping in response to the approach of an observer (White 1994, Fraser 1999). Anecdotally, *B. robustus* have been reported to seek refuge underneath rocks when disturbed by an observer, and to be highly elusive under certain weather conditions and at various times of the year (White 1994). Together, these observations lead to the conclusion that visual detection of individuals during monitoring events is likely to be highly imperfect. As such, it remains unclear whether the current monitoring method is adequate for making inferences about population trends, and it is unlikely to be an appropriate choice for post-release monitoring during a conservation translocation of *B. robustus.*

1.3.1. The first conservation translocation of B. robustus

The current threat status of *B. robustus,* along with overall trends of population decline mean the species requires immediate conservation action to secure its long-term persistence. In a combined effort between the Department of Conservation, Environment Canterbury and the University of Canterbury, the first translocation of *B. robustus* took place in February 2015. The translocation involved the collection of 216 individuals sourced from five wild populations. The grasshoppers were released into six 15 m x 15 m purpose-built gravel receiving habitats (36 individuals into each; T. Murray, unpub. data). Three of the receiving habitats were established within a pre-existing mammalian predator reduced area at the kakī/black stilt (*Himantopus novaezelandiae*) captive breeding centre located near the town of Twizel in the Mackenzie Basin, and the other three were established immediately adjacent to this area where mammalian predator presence was not controlled. All receiving habitats were spaced > 65 m apart. The purpose of this translocation was to explore translocation and monitoring methods to improve future translocation success for *B. robustus* and other insect species.

1.4. The current study

The general aim of this thesis is to generate knowledge required to develop applied solutions for improving future translocation success for *B. robustus*, and to advance the field of insect conservation science by contributing knowledge, methods and tools that can be applied or adapted for conserving threatened insects more broadly. The research included in this thesis began in the summer following the initial translocation of *B. robustus* (2015-16). It focuses on four key research objectives, each related to an aspect of the translocation procedure where development could enhance success. The four research objectives are:

1. To understand the life history of *B. robustus.*
2. To identify habitat requirements for *B. robustus.*
3. To determine whether *B. robustus* is threated by introduced mammalian predators.
4. To develop effective monitoring protocols for *B. robustus.*

Each chapter in this thesis focuses on one of the research objectives identified above. Each chapter is presented as a manuscript prepared for submission to a peer reviewed journal, and includes an Abstract, Introduction, Methods, Results, Conclusions and/or Discussion and References section. A brief description of the research objective considered in each chapter is presented below, however detailed rational is included in the Introduction of each chapter. In addition, each chapter is fronted with a preface that provides context for the research objectives within the aim of the thesis.

Chapter 2: The life history of *Brachaspis robustus* with implications for conservation management

The research objective of this chapter is to determine life history traits, and the biological requirements for development and reproduction of *B. robustus.* Grasshoppers were held in captivity in both a laboratory and field environment and observed for the duration of their life span. The implications of this research for improving conservation translocation and management success for *B. robustus* are discussed.

Chapter 3: Using radio telemetry to reveal movements of a Nationally Endangered grasshopper in two contrasting habitats with implications for conservation management

This chapter investigates the habitat requirements of *B. robustus* adult females. Using radio transmitters attached to their pronotum, adult female grasshoppers were tracked for up to 11 days in an open braided river, and in a modified gravel habitat. By comparing movements in the natural and

modified landscapes, key habitat attributes that could improve conservation translocation success are identified.

Chapter 4: Evidence that reducing mammalian predators is beneficial for threatened and declining New Zealand grasshoppers

The research objective of this chapter is to determine the threat that introduced mammalian predators pose towards the persistence of dryland grasshoppers including *B. robustus*. This chapter builds directly upon the conservation translocation of *B. robustus* in early 2015 by comparing the persistence of translocated populations in the predator reduced area and the non-predator reduced area. In addition, long-term trends of three populations of another dryland grasshopper species, *Sigaus minutus*, that occur in areas of varying predator control intensity, were assessed.

Chapter 5: Informing long-term monitoring protocols for a highly cryptic Nationally Endangered insect: Removal sampling as a basis for protocol development

Chapter 5 is the first of two chapters that work towards developing an effective monitoring protocol for *B. robustus*. In this chapter, a focus is placed on rapidly enhancing monitoring guidelines using data collected intensively within a single active season (between November and March). The research in this chapter leads to several suggestions for improving the effectiveness of a monitoring strategy and provides an example of how a monitoring strategy could be rapidly developed for other less-studied insect species.

Chapter 6: Designing monitoring protocols to measure conservation benefits for a highly cryptic threatened grasshopper

Chapter 6 is the second of two chapters that work towards developing an effective monitoring protocol for *B. robustus.* Using a three-season long dataset, this chapters builds directly upon the findings of Chapter 5 by addressing most of the gaps identified therein. Where Chapter 5 provided general enhancements to the monitoring protocol, Chapter 6 delves into the specifics of monitoring frequency, timing within the season, and design parameters to produce an applied strategy suitable for monitoring post-translocation success and long-term population trends.

Chapter 7: General Discussion

The final chapter of this thesis draws conclusions from the key findings of the research presented herein to inform how best to improve conservation translocation success for *B. robustus* and discusses the contributions this research has made to the advancement of insect conservation science.

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Chapter 2 The life history of *Brachaspis robustus* and its implications for conservation management

2.1. Preface

The focus of the first research chapter in this thesis is to develop an understanding of the life history of *B. robustus.* Having a thorough understanding of a threatened insect's life history traits and biological requirements for growth and reproduction underpins successful conservation translocations in a multitude of ways. Some examples include identifying individuals that are at reproductive age and therefore suitable for including in a founder population, understanding or estimating post-release population trends based on life expectancy and reproductive output, or informing parameters that are required for some models that can inform conservation decision making. Here, *B. robustus* were observed using large *in situ* field cages, and smaller *ex situ* cages in a laboratory to determine life history parameters including life expectancy, reproductive output of females, and the sex ratio and survivorship of offspring.

Sections of this chapter will be prepared into a manuscript to be submitted to *The New Zealand Entomologist*.

The life history of *Brachaspis robustus* and its implications for conservation management

2.2. Abstract

A thorough understanding of a threatened insect's life history traits and biological requirements for growth and reproduction are critical for achieving conservation translocation success. However, for many threatened insect species, the extent of life history knowledge is limited, and key information required to inform conservation decision making is missing. This is the case for the robust grasshopper (*Brachaspis robustus*), a Nationally Endangered insect endemic to the Mackenzie Basin of New Zealand. Large *in situ* field cages were used to observe *B. robustus* for the duration of their life cycle under natural conditions. Smaller *ex situ* laboratory cages were used for closer observation of *B. robustus* development and reproduction, and to experimentally develop captive rearing protocols. In the field, the life cycle of *B. robustus* was observed to be ~27 months from when an egg is laid to the death of the resulting adult. Grasshoppers kept in the laboratory (at between 14 °C and 34 °C) matured approximately six months earlier than those in the field which experienced naturally cold winter conditions (between -6 °C and 27 °C). Females were observed to mate with multiple males and laid up to eight egg pods in their lifetime, although no more than three were observed to be laid under field conditions. Eggs went through a diapause during development which was almost certainly driven by cold winter temperatures. At least 50 % of the offspring from most egg pods were female, and survivorship of nymphs was low in both field and laboratory cages. This study contributes vital knowledge required to inform conservation translocation and management decision making for *B. robustus*, and protocols for improving captive rearing success.

2.3. Introduction

A thorough understanding of a species' life history traits and biological requirements for growth and reproduction are critical for achieving conservation translocation success for threatened insects. For example, knowledge of biological requirements is vital for informing captive rearing protocols (Honan 2008) should translocations require larger founder populations than can be sourced from the wild (Pearce-Kelly et al. 2007). Similarly, knowledge of biological requirements is necessary for identifying receiving habitats that provide resources for growth and reproduction (McGrath et al. 2017). Life history traits are also required as input for key conservation decision making tools. For example, the simulation software *AlleleRetain* requires a number of user-defined parameters such as the lifespan of the species, the annual number of offspring per individual, and the proportion of offspring that are male, to determine the optimal number of founders to maximise the retention of genetic diversity over time (Weiser et al. 2012). Often understanding of life history traits and biological requirements for growth and reproduction is limited or missing for threatened insect species (Honan 2008, Braby 2018), and presents a knowledge gap that can critically hamper the success of a conservation translocation.

The Nationally Endangered robust grasshopper (*Brachaspis robustus*), a short-horned grasshopper endemic to the Mackenzie Basin of New Zealand (Townsend et al. 2008, Stringer and Hitchmough 2012, Trewick et al. 2014), has previously been excluded from life history research of New Zealand Acrididae grasshoppers because of its rarity (Hudson 1970). The species is a braided river specialist that prefers open rocky habitat. Key threats to its persistence include introduced predatory mammals (Chapter 4) and habitat degradation including the encroachment of exotic weedy species into braided riverbeds (Chapter 3, O'Donnell et al. 2016). Conservation management action for *B. robustus* could include conservation translocation to areas where predator control is currently implemented for threatened bird species (e.g. the braided river island in the Ōhau River that receives direct and high intensity mammalian predator control set up for the protection of black-fronted terns *Chlidonias albostriatus*; Woolmore et al. 2010) and may require a captive rearing for release programme to source founders for a translocation, or to augment translocated populations, or wild populations that are in steep decline. Knowledge about *B. robustus*' life history traits and biological requirements is crucial if these conservation management strategies are to be successful.

The life cycle of two other species within the genus *Brachaspis*, *B. collinus* and *B. nivalis*, are reported to be unsynchronised and semivoltine (Batcheler 1967, Mason 1971). Generally, *B. collinus* lay eggs in late December which then hatch the following spring (Batcheler 1967). The nymphs then develop through the summer and over-winter as late instar nymphs (instar six or seven), becoming

adults and mating the following summer (Batcheler 1967). Both *B. collinus* and *B. nivalis* females have been observed laying multiple egg pods in a laboratory environment, although most only laid a single egg pod before dying, and there have been no observations of multiple egg pods laid in the field (Mason 1971). Mason (1971) also observed that some females continued to develop subsequent eggs within their ovaries into autumn and speculated that low fat reserves during winter led to starvation and death. Eggs for both species enter obligatory diapause during development that is thought to be terminated by exposure to a period of cold, likely below 0 °C, although the exact requirements were not experimentally confirmed (Mason 1971). Both species have flexible life cycles in that eggs, juvenile instars, and adults all have the ability to survive over winter (Batcheler 1967, Hudson 1970, Ramsay 1978).

The first and only description of the *B. robustus* life cycle (White 1994) is based on evidence recorded during monthly visits to a single population over two summer periods. Observations of overwintering juveniles suggest a semivoltine life cycle (White 1994). The occurrence of different life stages throughout the season imply *B. robustus* lacks synchronisation (White 1994) and has a flexible life cycle similar to that of *B. collinus* and *B. nivalis.* However, both *B. collinus* and *B. nivalis* are alpine species found at 1,000 – 1,800 m a.s.l. (Hudson 1970), whereas *B. robustus* is usually found below 800 m a.s.l. (Trewick 2001). The difference in the species' altitudinal ranges is likely to result in several differences in life cycle parameters because the alpine species will generally experience longer, colder winters than *B. robustus.* Late spring snowfall and year-round frosts can occur within the Mackenzie Basin, however the weather usually has distinct seasons with highs of > 30 °C in the summer and winter lows of < -10 °C (Macara 2016). All three species inhabit gravel environments, however *B. collinus and B. nivalis* both inhabit alpine screes (Hudson 1970) whereas *B. robustus* inhabits the gravel beds and terraces associated with braided rivers (Bigelow 1967, White 1994, Fraser 1999). Braided rivers are high-disturbance environments characterised by highly variable flows and flooding events that often change channel morphology and braid dynamics (Gray and Harding 2007), and their unpredictable nature could also have implications on life history parameters and population dynamics.

In this study, large *in situ* field cages were used to observe *B. robustus* growth and reproduction under natural conditions in the absence of predation by birds and mammals. We also used smaller *ex situ* laboratory cages for closer observation of *B. robustus* development, and to experimentally establish captive rearing protocols. The aim of the current study is to determine life history parameters including lifespan, reproductive output and sex ratios of offspring that are required as input into *AlleleRetain*, and determine requirements for development and reproduction of *B. robustus* for the purpose of informing future conservation management decisions and improve conservation translocation outcomes.

2.4. Methods

The two methods described below were used to track the reproductive output and development of *B. robustus* over three consecutive summers (November - January, 2015 - 2018). To add clarity to the experimental procedure, a timeline of events is presented in Appendix A.

2.4.1. Captive rearing in the field

2.4.1.1. Patersons Terrace

In January 2015, three field cages (Figure 2.1) were constructed to investigate the development of *B. robustus* nymphs and to test whether the cage design could withstand the conditions present in the Mackenzie Basin during the summertime (in particular, strong northwest winds and harsh ultraviolet light). The three cages were deployed at Patersons Terrace[2] (Figure 2.2) where a relatively large wild population of *B. robustus* occurs (Morris 2005) on an un-used gravel road laid during the 1970s for the construction of hydro-electric dams (McKay et al. 1978). A 3 m x 1.4 m wooden framed base (150 mm x 25 mm, treated pine) was placed in a shallow (5 - 10 cm) trench dug out in the stony substrate. Three 2.4 m x 6.8 mm Ø flexible fibre glass rods (Polynet Products Ltd, Christchurch) spaced 1.4 m apart were inserted along its length to form a curved roof, over which insect mesh (Biomesh, aperture 0.28 mm x 0.78 mm, Redpath New Zealand) was fastened with wooden battens screwed to the outside of the base. Two agricultural feed sacks were filled with large rocks and fastened to the wooden frame underneath the battens to anchor the cage during strong winds. Two guy wires (Warrior utility galvanised tie wire, 0.9 mm Ø; or, Ropestar braided wire galvanised steel, 3 mm Ø) were tied around the fibre glass rods at each end and passed through small holes in the insect mesh that were reinforced by a plastic fabric clip (Cosio polythene clips, 0.04 mm x 0.11 mm, butterfly connector snipped off). The end of each guy wire was anchored to the ground by a pile of large rocks outside the cage. Local riverbed sand (sourced from Whitestone Contracting Limited, Twizel) was used to fill in the inside edge of the trench to ensure that there were no gaps for the grasshoppers to escape through. Observer entry into the cage was by unscrewing the bolts on the short edge of the cage, removing the baton, and lifting the insect mesh. Clamps (Fuller F-clamp or G-clamp) were used to fasten excess insect mesh on the observer entry end (Figure 2.1).

[2] Patersons Terrace is also commonly known as the Tekapō Triangle.

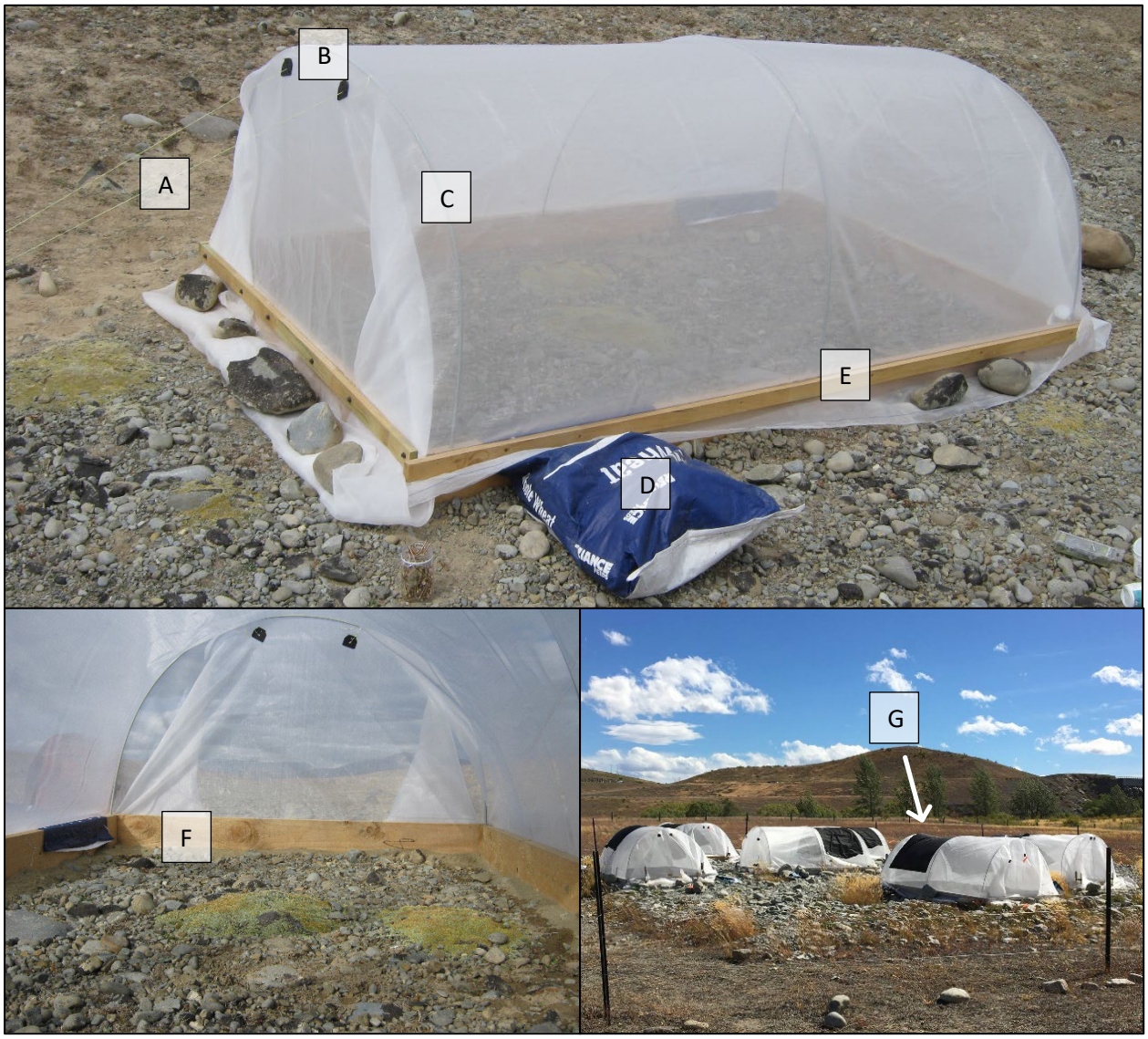

Figure 2.1. Large field cage constructed to hold grasshoppers for observation *in situ* at Patersons Terrace (top) showing (A) guywires, (B) plastic fabric clips, (C) internal fibre glass rod, (D) agricultural feed-sack filled with large rocks, (E) wooden batten. Inside view of *in situ* field cage over established *Raoulia australis* plants (bottom left) showing (F) wooden-framed base. Six cages constructed on a purpose-built gravel patch near at the kakī aviary complex with the addition of the black shade cloth (G) attached to the outside of the cage (bottom right).

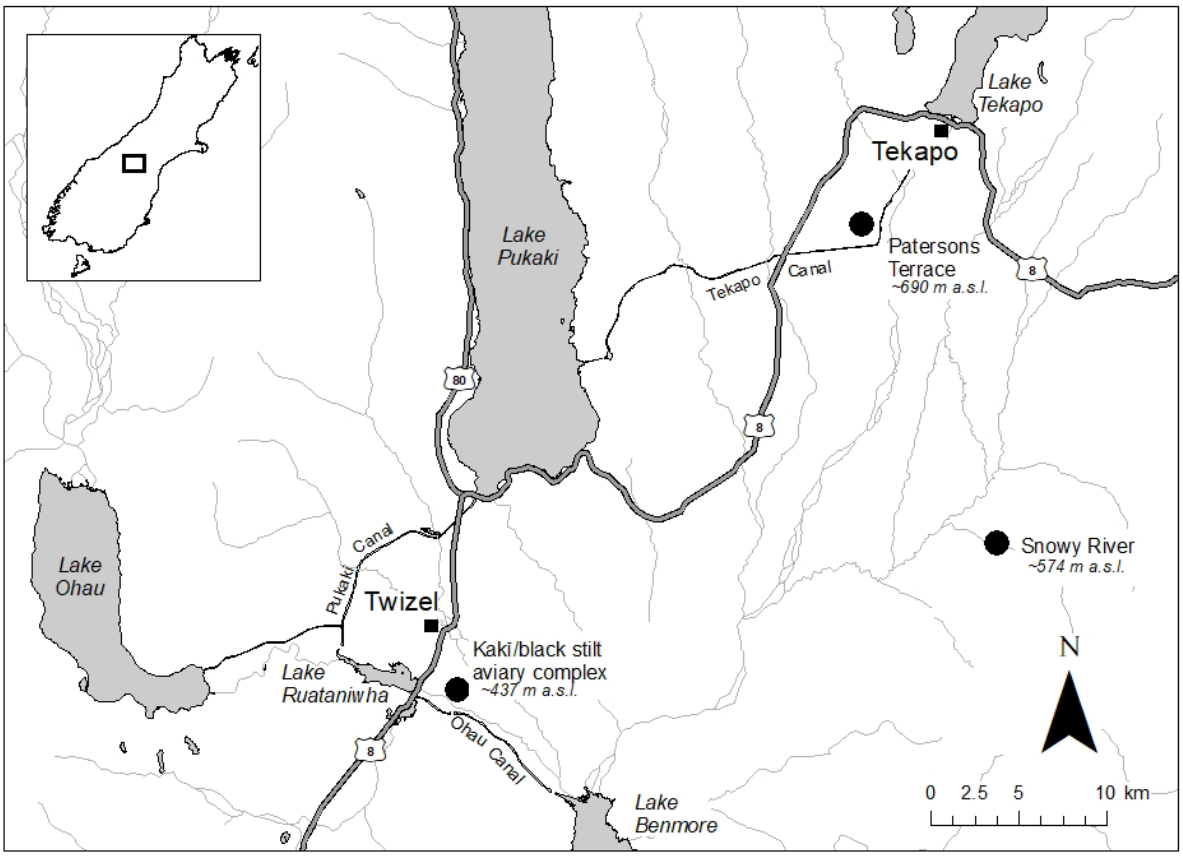

Figure 2.2. The locations of Patersons Terrace, Snowy River and the kakī aviary complex in the Mackenzie Basin, South Island, New Zealand (inset), where *B. robustus* were collected from and observed in field cages.

On 21/01/2016, five wild nymphs were released into each of the three large cages. Within each cage, individual nymphs were marked with a different coloured dot on the pronotum using a non-toxic paint pen (Edding® 780 gloss paint marker). Nymphs were collected from habitat in the immediate vicinity of each cage. Fewer female nymphs were found than males, resulting in 10 males (body length 9 – 12 mm, femur length 5 – 6 mm) and 5 females (body length 9 – 13 mm, femur length 5 – 6 mm) being captured in total. Each fortnight, femur length and body length (from top of head to tip of abdomen) were measured for each grasshopper, and identification marks were replaced if lost as a result of moulting. Attributes such as body colour or missing hind legs were used to identify individual grasshoppers to determine the appropriate colour mark in situations where more than one individual had moulted between monitoring events. Nymphs were kept within the cages until the end of March 2016 when the cages were removed.

A further four small cages (BugDorm white headless emergence traps, L60 x W60 x H60 cm) were set up on the gravel road in December 2015. A single adult female and adult male, sourced from the surrounding habitat, were placed into each cage. Cages were situated such that some vegetation (e.g. *Raoulia australis*, hawkweed (*Hieracium* spp. and *Pilosella* spp.) and/or exotic grass species) was

growing inside, and grasshoppers were supplementary fed with excised hawkweed flowers. Each cage was supplied with a circular plastic tray (Ø11.8 x H12.5 cm) with drainage holes drilled in the base filled with sterilised sand and buried so that the lip of the tray was level with the ground. No offspring were found in the cages within the monitoring period (November 2015 – March 2016). Because the cages were not designed to withstand winter snowfall, the footprints of each cage were marked with large yellow tent pegs and the cages were removed at the end of March 2016. Any remaining adult grasshoppers were released. In November 2016 the cages were again set up in the exact location of the previous summer and checked weekly for any nymphs emerging from eggs laid in the gravel in the previous summer.

2.4.1.2. Kakī aviary complex, Twizel

To facilitate more regular access to observe grasshopper development, the three large cages were relocated to a gravel plot at the kakī aviary complex near Twizel in November 2016. The gravel plot (15 m x 15 m) was constructed in 2014 to receive translocated grasshoppers (Chapter 4, T. Murray, unpub. data). No grasshoppers had been observed in the plot since February 2016 despite regular monitoring (J. Schori unpub. data). Three additional large cages were built on-site, with the design modified to include a piece of black cloth (Kiwi Garden non-woven weed mat) attached to the outside of the insect mesh to provide shade inside half of the cage (Figure 2.1G). Two HOBO® H21-002 micro station loggers were used to record air temperature and relative humidity (HOBO® S-THB-M008 smart sensor) ~20 cm above ground surface, and temperatures on, and ~3 cm below, the ground surface (HOBO® S-TMB-M006 smart sensors). One station was set up inside of a cage, and the other on the gravel outside of the cages. Both recorded data at one-minute intervals from January to July 2017.

Each cage housed one adult male and one adult female between November and March. In 2016-17, four of these pairs were sourced from Snowy River and two from Patersons Terrace. The females were kept in a single cage for the entire summer (November – March) and the males were rotated (approximately every fortnight) between cages containing females from the same source population. Vegetation had established in the gravel plot since it was created in 2014, however cages were also provisioned with a turf (approximately 40 cm x 40 cm) bearing a mix of native and exotic plants, mosses and lichens sourced from the surrounding grounds. The turfs were assessed for desiccation several times a week and replaced as required. A large oviposition tray consisting of an open 2L ice cream container with 6 holes drilled into the bottom surface to allow for drainage and filled with sterile sand was provided in each cage. Trays were buried so that the lip of the tray was level with the surrounding gravel.

At the end of March 2017, the gravel within the cages was dug over by hand to search for egg pods. When an egg pod was found it was taken to the laboratory and the stones attached to the outside of the egg pod (Figure 2.3A) were removed under a binocular microscope (10 x magnification) using forceps (Figure 2.3B). The eggs were counted then the pod was placed in a plastic cup filled with gravel at a depth of 3-4 cm. The cup had holes drilled into the bottom of it to allow rainwater to freely drain and was buried in March back into the gravel patch at the kakī aviary complex with the top lip of the cup level with the ground surface. The eggs were kept in the ground over winter.

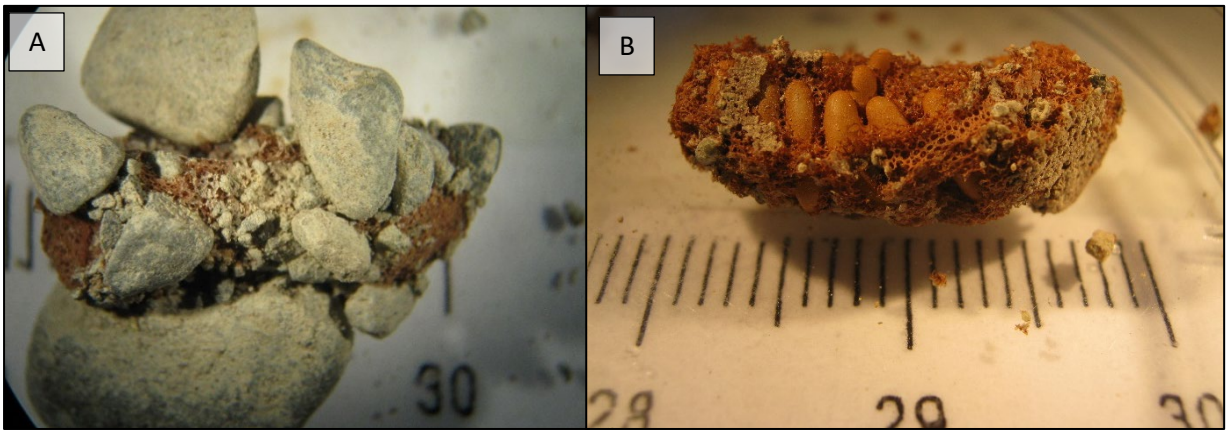

Figure 2.3. The first egg pod discovered in a large field cage holding a pair of *B. robustus* at the kakī aviary complex. (A) the egg pod as it was found in the field, (B) the egg pod after stones had been removed to reveal the individual eggs inside. Gradations = mm.

In November 2017, two egg pods were transferred to the laboratory at the University of Canterbury in Christchurch. Both pods were laid by Snowy River parents. They were kept in the gravel filled cups on a laboratory bench with mesh bags over top to contain any emerging nymphs. Within 6-12 hours of hatching nymphs were moved into BugDorm-1 insect rearing cages (W30 x D30 x H30 cm). Each time a grasshopper moulted the exuviae was collected. When nymphs were large enough to handle safely, individuals were sexed, and males and females divided into separate cages. In May 2018, females were separated into their own cages with one or two males for mating. The methods for keeping grasshoppers in the laboratory are described in section 2.4.2.

Nymphs that hatched within the field cages were first counted in December 2017 when they were large enough to handle, and again in March 2018. In October 2018, they were removed as late-instar juveniles from the field cages and brought back to the laboratory to be reared to adulthood. In November 2017, six new pairs of adult grasshoppers (three pairs sourced from Snowy River and three from Patersons Terrace) were placed into the cages and maintained until the end of March 2018 following the same methods as described above.

2.4.2. Captive rearing in the laboratory

In late October 2017, wild late-instar *B. robustus* were collected from Patersons Terrace (2 x females, 2 x males) and Ōhau River (3 x females, 3 x males) and brought into the laboratory at the University of Canterbury. Adult females were put into individual rearing cages (BugDorm-1,W30 x D30 x H30 cm) with a male from the same source population. All cages housing grasshoppers were kept on the laboratory bench at ambient room temperature (between 14 °C and 34 °C) and exposed to ambient sunlight. Heat lamps (Reptile One®, Daylight Halogen, 72W) were used to provide basking temperatures and UVA light during the day. Each cage was provided with rocks for basking and misted moss sourced from the Ōhau River for moisture. The grasshoppers were given a mixed diet of dandelion and daisy flowers sourced from the University of Canterbury campus, live seedlings grown in the greenhouse (including; spinach, cos lettuce, yarrow, borage, *Phacelia* sp., herbal-ley, carrot, forget-me-not, dandelion, clover, hawkweed) with the root mass and soil secured in a clear plastic bag closed with a zip-tie, and turfs bearing native and exotic vegetation sourced from the kakī aviary complex (only in 2016-17) presented in an aluminium tray. When dandelion flowers were not available, a small plastic dish of raw *Pinus radiata* pollen was provided as a source of protein. For mating pairs of grasshoppers, an aluminium dish (L17 x W9 x H4 cm) filled with coarse sand was provided in each cage for females to oviposit. Any eggs laid were removed from the sand and stored at ambient room temperature in a glass vial filled with coarse sand covered with a mesh lid.

To understand the conditions required for egg development, the eggs laid in the laboratory were divided into groups and allocated to one of four different conditions from the start of May 2018; constant cold, refrigerated at ~3.7 °C (*n* = 3); constant warmth, incubated in a growth cabinet at 15 °C (*n* = 3); warm fluctuating, ambient room temperature on a laboratory bench (*n* = 3); and control, egg pods (inside of gravel-filled plastic cups, as described above) were buried in the gravel at the kakī aviary (*n* = 10). At the end of October 2018, all egg pods were returned to the laboratory and placed in a growth cabinet on a cycle of 22 °C for 14 hours and 15 °C for 10 hours per day with a corresponding 14L:10D photoperiod.

2.5. Results

2.5.1. Copulation and oviposition

Female *B. robustus* were observed copulating in the field between November and March across all three study seasons, and for the duration of adulthood regardless of season in the laboratory. Females in the field and in the laboratory were observed copulating with different males as the males were rotated around the cages. No eggs were found in the sand trays provided in the field cages, but in the laboratory environment females readily oviposited in the coarse sand provided. In the field cages, eggs were generally found in the gravel that formed the base of the cages, but in one instance an egg pod was found among a plant root base. Oviposition was observed both in the laboratory and in the field on multiple occasions but always midway through the process so the complete duration of an oviposition event was never recorded (Figure 2.4).

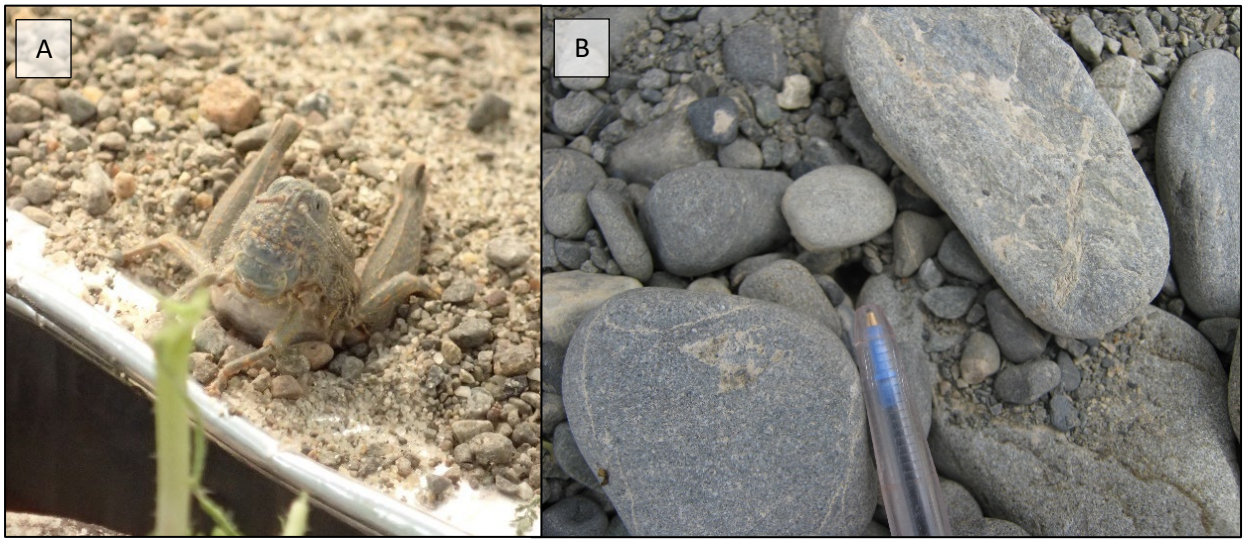

Figure 2.4. (A) *B. robustus* ovipositing in an aluminium dish of coarse sand in the laboratory (Photo: T. Murray). (B) A hole left by an ovipositing female in the gravel of a large field cage at the kakī aviary complex. The site of oviposition was not normally visible, and for all other observations once the female removed her abdomen from the ground the surrounding gravel and sand fell into the hole to conceal it.

2.5.2. Egg pods

In the large field cages at the kakī aviary during seasons 2016-17 and 2017-18, most females laid 1 (*n* = 5 females) or 2 (*n* = 4 females) egg pods, but one individual produced 3, and two produced none.

The mean number of eggs per pod was 26 (min = 17, max = 35). Grasshoppers kept in the laboratory laid up to 8 egg pods in their lifetime, and their individual eggs measured between 8.9 mm and 10 mm in length.

Egg pods laid in the field were observed to be particularly delicate when the ground was damp after rainfall and under those conditions they frequently fell apart when they were retrieved for counting. In dry conditions, egg pods were able to be extracted intact. In the summer of 2017-18, some of the egg pods retrieved for counting were found to contain collapsed eggs, empty or broken eggs shells, and several others had a fungus growing on them (Figure 2.5). It is unclear whether the empty eggshell eggs were laid the previous summer and had already hatched, or whether the eggs had suffered from predation. The former seems unlikely given there were no nymphs in the cages that were unaccounted for, but it is possible that hatching might have occurred prior to field cage construction. Two types of mites were observed in association with the eggshells. The first was a large, dark mite thought to be from the family Caeculidae (Figure 2.5B). They are generally ambush predators of small arthropods but were more likely scavenging on dead eggs in this case (M. Shaw, pers. comm.). The second type was a small, translucent mite (Figure 2.5E) thought to be from the family Acaridae, which are generally scavengers (M. Shaw, pers. comm.).

Figure 2.5. *B. robustus* eggs and associated mites retrieved following over-wintering in large field cages at the kakī aviary. (A) Fungal growth on *B. robustus* egg. (B) A mite thought to be from the family Caeculidae. (C) Empty eggshells within a pod of eggs. (D) A collapsed egg (indicated by arrow) among intact eggs and fungi from a pod of eggs that fell apart upon retrieval. (E) Mites thought to be from the Acaridae family.

2.5.3. Timing of hatch and sex ratios at emergence

No eggs hatched within the same summer that they were laid, and those that hatched only did so after overwintering under natural winter conditions in the ground of the Mackenzie Basin. Eggs laid in the cages at Patersons Terrace (~690 m a.s.l.) in the spring/summer of 2015-16 began hatching on 6/12/2016, and three from four of the small cages contained nymphs by 30/12/2016. No nymphs were ever observed in the fourth cage. Because 1st instar nymphs are very small (~6 mm body length, ~3 mm femur length), it was not possible to count nymphs within the field cages until several weeks after hatching during which time the fabric of the small cages became severely degraded and large tears appeared in the fabric. Although many of the nymphs escaped, a minimum of 14, 18 and 26 nymphs were counted respectively in three of the four cages before the small cages were removed and any remaining nymphs were released.

At the kakī aviary complex (~437 m a.s.l.), hatching occurred between 03/11/2017 and 13/11/2017 in the summer of 2017-18. This was earlier than anticipated (early November, compared to early December the previous year at Patersons Terrace), and although hatched nymphs were contained within the large field cages, small mesh bags had not been placed over the individual egg cups to allow accurate nymph counts at hatching. For all egg pods that hatched in the field, ≥ 50 % of the nymphs were female (mean of 68 % female) at first count several weeks after hatching. Survivorship appeared relatively equal between the sexes. Males outlived females from two pods, females outlived males from two pods, and the remaining three pods showed mixed survivorship ratios (Figure 2.6).

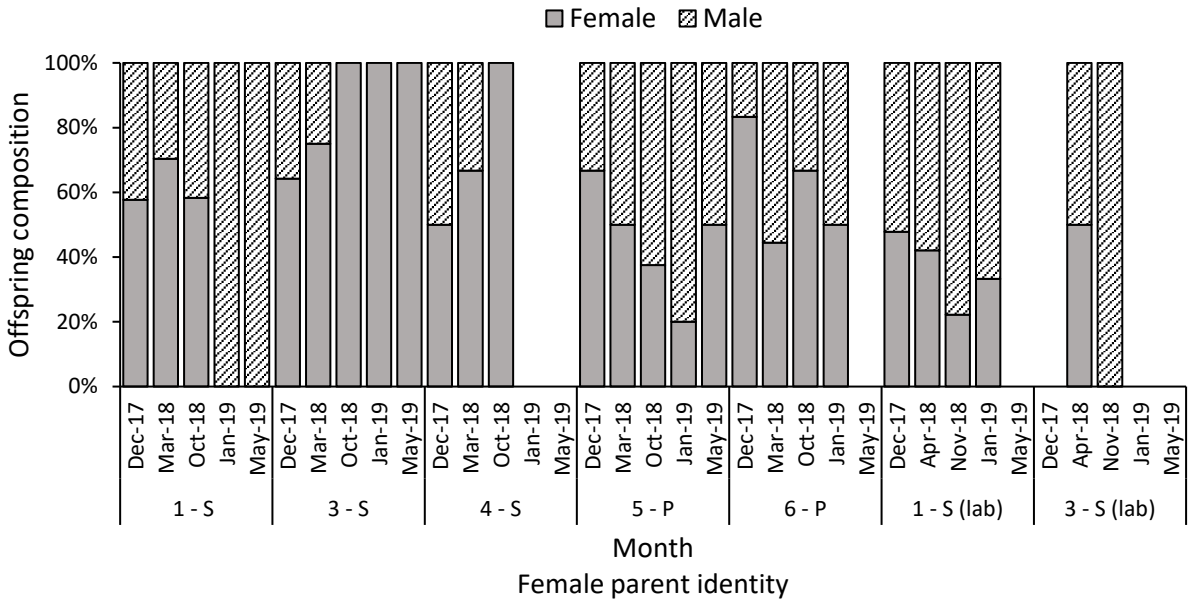

Figure 2.6. The sex ratios of nymphs that hatched from eggs laid by five females in large field cages during the summer of 2016-17 at the kakī aviary complex. Females 1, 3 and 4 originated from Snowy River ('S'), and females 5 and 6 originated from Patersons Terrace ('P'). Two of the egg pods ('1-S (lab)' and '3-S (lab)') were hatched and raised in the laboratory at the University of Canterbury. Nymph sex was first assessed approximately four weeks after emergence (except for '3-S lab') when nymphs were large enough to observe.

The two egg pods that had been relocated to the University of Canterbury laboratory in October 2017 hatched 32 and seven nymphs beginning on the 03/11/17 and the 05/11/17 respectively. All nymphs emerged above the gravel within five days of the first nymph. The following summer, only a single nymph hatched from the eggs that were relocated to the growth cabinet at the laboratory. It hatched on 13/11/2018. Because no further hatching was observed for > 8 weeks the remaining pods were inspected. Approximately half the eggs in a pod that had been returned from overwintering under field conditions had hatched, but the vermiform were found dead and buried at the bottom of the container. For all other treatments, no hatching had occurred.

The number of nymphs declined rapidly in both the large field cages and the small laboratory cages (Figure 2.7) but because few bodies were found it could not be determined whether nymphs had died or escaped. During the collection of the remaining grasshoppers from the large field cages in October 2018, after overwintering as nymphs, two bodies were found that appeared to have died from a fungal infection. Histological examination of a further two individuals that subsequently died in the laboratory confirmed they were infected with an entomopathogenic *Beauveria* fungus (T. Glare, pers. comms.).

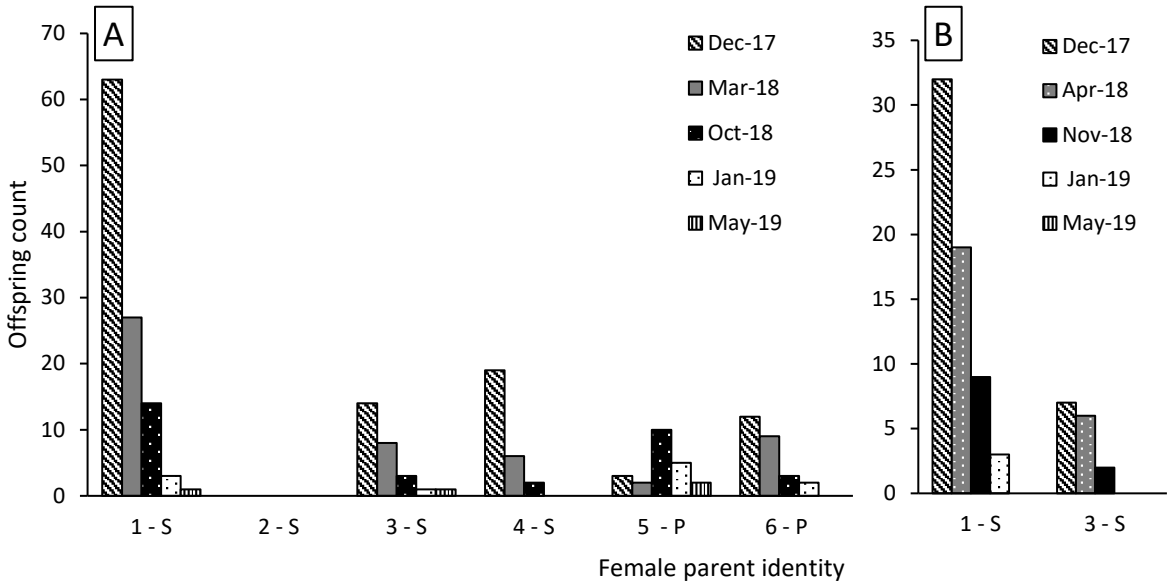

Figure 2.7. The approximate survivorship of nymphs that hatched from the egg pods laid in large field cages at the kakī aviary complex in Twizel in 2016-17. (A) Nymphs that were raised in the large field cages. (B) Nymphs that were raised in the laboratory. Female parents 1 to 4 were sourced from Snowy River (S) and female parents 5 and 6 were sourced from Patersons Terrace (P). Female 2–S did not produce any eggs. Counts are approximate, and sometimes increased over time (i.e. 5-P) because small early instar nymphs could not be detected on all occasions.

2.5.4. Development and longevity

Males and females were observed to pass through at least five instars, although the exact number of instars to reach adulthood was not determined. In the laboratory environment, 85 % (*n* = 10) of males and 18 % (*n* = 2) of females had reached adulthood within 22 weeks of emergence. The longest a male took to reach adulthood was 34 weeks. In contrast, nymphs raised in the large field cages that were exposed to natural cold winter temperatures had still not reached adulthood by 51 weeks after emergence even though they hatched at the same time as those raised in the laboratory. Grasshoppers hatched in the field and brought into the laboratory after winter out lived those hatched and raised in the laboratory (laboratory hatched; oldest male died 61 weeks after hatching, female at 69 weeks, field hatched; male at 86 weeks, female at 87 weeks). The age of the adult mating pairs of grasshoppers initially placed in the field cages was unknown at the time of their capture. However, only one female of the 24 grasshoppers kept in the large field cages during this study lived beyond the end of March. She did not survive the winter, dying sometime in April or May.

2.6. Discussion

2.6.1. Copulation and oviposition

The highest number of egg pods laid by an individual *B. robustus* female in the field was three, compared to eight in the laboratory. This may have been influenced by the fact that grasshoppers in the laboratory were held in captivity from the time they reached adulthood until death, whereas the ages of wild females placed in the field cages were not known, and they were only held in captivity until the end of March each year. Therefore, the observation period for laboratory females was longer than for field females, and it was not known whether the wild caught females had already laid egg pods prior to capture.

Another factor that can determine the number of egg pods laid is temperature (Willott and Hassall 1998). In this study, females in the laboratory were exposed to relatively warm temperatures every day, compared to those in the field which were exposed to naturally fluctuating temperatures through the seasons. The higher number of eggs laid under such laboratory conditions is consistent with the findings of Willott and Hassall (1998) who reported that raising temperature by 5 °C increased the number of egg pods laid within the life time of *Chorthippus brunneus* and *Stenobothrus lineatus*, and reduced the time between pods being laid by *C. brunneus, Omocestus viridulus* and *Myrmeleotettix maculatus*.

Diet can also influence reproductive output. For example, higher availability of nitrogen (Joern and Behmer 1997) and plant species richness (Unsicker et al. 2010) has been shown to increase production of egg pods. Grasshoppers kept in the laboratory had access to plants *ad libitum*, while grasshoppers in the large field cages had to forage more widely and had limited access to flowers, which likely provided a good source of protein, lipids, and amino-acids (Nicolson 2011).

Another factor that could affect the production of egg pods is the number of mating events a female is exposed to (Zhu et al. 2013). Secretions from the male accessory gland passed on during mating can stimulate egg production (Pickford et al. 1969), but can also reduce female attractiveness to subsequent mates, hence increasing the time between mating events (Gillott 2003). However, more mating events also increases the availability of sperm to fertilise subsequent egg pods. Parthenogenesis has been reported for some species of acridids (Wang and Sehnal 2013) but could be neither confirmed nor rejected for *B. robustus* in this study because all females were given the opportunity to mate. Mate availability *per se* is unlikely to be an explanation in this study because there was no difference in the availability of mates for females kept in the laboratory cages versus the large field cages.

Although preference testing to determine the optimal substrate for egg laying was not part of this study, females in the laboratory were observed to oviposit in trays of coarse sand provided in the cages indicating this is a suitable substrate to use in captive rearing. One female also oviposited between several large pebbles on the cage floor prior to sand trays being provided. In the field cages, grasshoppers always laid their eggs in the coarse gravel of the cage floor and never in the sand provided, however, the area of gravel available was much larger than the relatively small container of sand. As no *B. robustus* eggs have previously been collected from the wild, the substrate preference for oviposition has not been documented. However, F. Thorsen (unpub. data) reported observing two grasshoppers ovipositing in gravel areas at Snowy River. It is possible that grasshoppers prefer to lay eggs in gravel rather than sand because it provides greater protection during flooding or submergence events. For example, in Germany egg pods of the riverbed grasshopper *Bryodema tuberculata* are resistant to being submerged provided that the structure of the gravel is not disturbed (Reich 1991). Further research conducted in a natural environment could reveal preferred substrate for oviposition.

2.6.2. Egg development

Given that *B. robustus* eggs were only observed to hatch after being exposed to natural winter thermal fluctuations while buried in the ground within their natural range, it seems likely that eggs require a cold period, presumably < 0 °C, to facilitate hatching. Obligate egg diapause that is broken by thermal cues occurs in other New Zealand acridids (Northcroft 1967, Mason 1971) and is probably critical for ensuring hatching does not occur during the harsh winter conditions of the New Zealand alpine regions. The observations from this study indicate that *B. robustus* egg development might also have an obligate diapause that prevents hatching from occurring during the harsh Mackenzie Basin winter. Once the conditions for entering and breaking diapause are met, then the onset of warm spring conditions likely drives the timing of hatch. Previous observations have noted that *B. robustus* in the central basin were two to three weeks further advanced than those further north at higher altitudes (White 1994). In the current study, nymphs hatched a month earlier at the kakī aviary complex (~437 m a.s.l.) than at Patersons Terrace (~690 m a.s.l.) which is ~250 m higher elevation and warms slightly later in the season.

The conditions required for egg hatching appear to be met over the current altitudinal range of the species at present. However, if milder winters and fewer frost days become more common as a result of climate change (Plummer et al. 1999, Easterling et al. 2000, Tait 2008) then necessary thermal cues may not be met, and populations of *B. robustus* could become extirpated from lower elevation sites such as the Ōhau River. Further controlled experiments to determine the temperature

thresholds and thermal accumulation required to initiate and break egg diapause in *B. robustus* will benefit the future management of the species. For example, when used in conjunction with climate change models the temperature thresholds for egg hatching could be used to predict future changes to *B. robustus* distribution, or suitable translocation receiving habitat that will support egg development and hatching. It is also possible that egg development could be accelerated in a laboratory environment once the thermal requirements are determined. The benefits of this include accelerated rearing of grasshoppers in captivity to support growth of wild populations for facilitating colonisation of more suitable habitat at higher altitudes.

2.6.3. Nymph development

Grasshoppers kept in captivity in the laboratory reached maturity approximately six months before the same cohort that was raised in captivity in the field. The differences in the rate of development between the two cohorts is likely to be driven primarily by temperature (Clissold and Simpson 2015) and partly by the availability of food resources (Bernays and Bright 2001). Grasshoppers kept in the laboratory were kept at warm temperatures for the duration of their development, whereas those kept in the large field cages likely entered quiescence, pausing development through the colder winter season and resuming development when temperatures warmed in spring. This would have retarded development of field grasshoppers compared to laboratory grasshoppers. Furthermore, food resources including flowers were provided *ad libitum* in the laboratory and were always in close vicinity to the grasshoppers because of the confined nature of the cages used. In comparison, grasshoppers in the large field cages had to forage more widely for resources. Even small increases in the distance to food resources (~20 cm) have been shown to decrease diet variety, that in turn prolongs development in acridids (Bernays et al. 1997).

Although *B. robustus* nymphs were observed to survive winter, it is unclear whether the species uses a freeze avoidant (super cooling of internal liquids to avoid the formation of internal ice crystals) or freeze tolerant (ability to withstand the formation of internal ice crystals) strategy. A freeze tolerant strategy with quiescence rather than diapause is common among southern hemisphere insects (Sinclair et al. 2003), and is advantageous because it allows insects to survive through unexpected cold periods at any time of year and also take advantage of mild days in winter to feed. Because active *B. robustus* have been observed on warm days in autumn and spring even after night-time temperatures < 0 ° C (pers. obs.) it seems likely that the species utilises a freeze tolerant strategy.

This study was unable to determine the number of instars that *B. robustus* pass through to reach adulthood, in part because the grasshoppers occasionally consumed their exoskeletons after moulting

making a *post hoc* count of collected exoskeletons less informative, and also because of the low percentage of grasshoppers kept in captivity that reached adulthood. However, it was determined that both sexes pass through more than five instars. The development of *B. robustus* is likely to be similar to that of other *Brachaspis* species such as *B. collinus* and *B. nivalis* which both pass through seven instars for females, and six instars for males (Hudson 1970). For some acridids, warmer temperatures can result in the addition of an instar in the moult before adulthood is reached (Willott and Hassall 1998) which could mean some variation in *B. robustus* development could occur dependant on summer weather conditions.

2.6.4. Causes of death

Offspring in the laboratory and in field cages had low survivorship despite the absence of mammalian and avian predators. It is possible that young grasshoppers continued to suffer predation events from spiders and other predatory invertebrates such as pseudoscorpions. Small invertebrate predators would have been present in the gravel in the large field cages and may have been introduced to laboratory populations through the soil of the turfs that were initially used for feeding. Predatory spiders are known to target young grasshoppers more than older grasshoppers in Arthur Country, USA (Oedekoven and Joern 1998), and likely accounted for some of the *B. robustus* nymph deaths here. No instances of cannibalism were observed for *B. robustus*.

Two deaths in the large field cages were caused by entomopathogenic fungi from the genus *Beauveria* (T. Glare, pers. comms.). *Beauveria* is commonly used as a mycopesticide for biocontrol of insects (Lomer et al. 2001), and was introduced to New Zealand in the nineteenth century (Cummings 2009). Although acridids are susceptible to entomopathogenic fungi, they can sometimes avoid infection by moving to a warmer microhabitat, or reduce the effects of infection by raising body temperatures during basking (Carruthers et al. 1992, Inglis et al. 1996). For example, *Beauveria bassiana* has a thermal threshold of > 8°C to < 37 °C (Fargues et al. 1997), but the surface temperatures of riverbed rocks in the Mackenzie Basin range between -6 °C in winter to 58 °C in summer (J. Schori, unpub. data), indicating that the prevalence of *B. bassiana* could be suppressed in wild *B. robustus* populations under certain conditions. It is possible that the large field cages provided favourable conditions for fungal infection by shading rock surfaces and keeping temperatures within the fungi thermal thresholds. It is also possible that the cage mesh diffused ultraviolet light making conditions more favourable for spore persistence compared to direct sunlight conditions in the wild (Inglis et al. 1995, Costa et al. 2001).

In the laboratory, individuals from the first pods of grasshoppers that hatched in November 2017 are likely to have been killed or lost due to the way food was provided and changed. The turfs bearing native food plants were presented in small aluminium containers, and nymphs may have become stuck or been hidden in the small gaps between the turf and the container edge and unintentionally discarded when the food was changed. No grasshoppers were lost when the feeding protocol was changed to provide grasshoppers with fresh grown food presented in small clear plastic bags.

For the egg pods that failed to hatch in the laboratory in November 2018, vermiform were found deceased near the bottom of the gravel cups for egg pods that had over wintered in field conditions. This suggests that the eggs hatched successfully, but the vermiform failed to orientate themselves to the surface for emergence. Diffuse light inside of the growth cabinet may have resulted in them orientating the wrong way, however, vermiform of other Orthopterans have been shown to be negatively geotaxic, and even if eggs are orientated upside-down, vermiform will navigate to the surface (Bernays 1971). Another possible explanation is that vermiform did not emerge from the surface because the gravel in the cups was unsuitable for digging and prevented them from surfacing.

Many other deaths occurred both in the laboratory and in the field cages for which the cause has not yet been identified. Mason (1971) described several agents affecting New Zealand alpine species of *Brachaspis* including Mermithids (family Nematoda), external mites, tracheal mites, cystiseroids and gregarines. There were no observations of mermithids affecting *B. robustus* in the laboratory nor in the field. Small numbers of external mites have been observed in wild populations of *B. robustus,* particularly at Ōhau River, and several occasions at Patersons Terrace (pers. obs.) but none were seen on the grasshoppers kept in the field cages, or in the laboratory populations. Dissection of deceased grasshoppers is planned to determine the presence of any parasites and disease in captive *B. robustus*. Mason (1971) also identified one egg parasite, a Hymenoptera from the genus *Scelio*. Another potential predator of Orthoptera eggs are the larvae of Bombyliidae flies (Lomer et al. 2001), of which only one species is found in New Zealand (Paramonov 1959). Fungi is also able to penetrate and destroy eggs and was observed on several *B. robustus* eggs found in the field cages during 2017-18, however it was not determined whether the observed fungi killed the eggs or were simply decay fungi.

2.6.5. Conservation implications

Several life history strategies that contribute to the survival *B. robustus* through harsh winters, variable climate, and in a high disturbance habitat were identified in this study. Eggs likely have an obligate

diapause that prevents them from hatching during winter, while nymphs have adapted to survive sub-zero temperatures that occur frequently throughout the winter and on occasion at other times of the year. If winters become milder due to predicted climate change (Tait 2008), there are several possible outcomes for *B. robustus*. As long as diapause conditions are still met, mild winters could speed-up development such that grasshoppers reach maturity earlier in the season (Bale and Hayward 2010). This could be positive because it could extend the growth and reproductive period for grasshoppers, resulting in more offspring. Milder winters could also increase the prevalence of disease in overwintering nymphs, and if diapause conditions are not met then milder winters could impede egg development and delay or prevent emergence.

This study has determined several key life history traits and biological requirements for *B. robustus* that will contribute to the improvement of conservation management for the species. This includes determining the life span of the species, the reproductive potential of individuals, and the sex ratios of offspring that are essential user defined parameters for the simulation software *AlleleRetain* (Weiser et al. 2012). This study has also identified biological requirements for growth and reproduction, and developed protocols for captive rearing in the laboratory environment which has important applications if a captive rearing strategy is required for augmenting wild or translocated *B. robustus* populations. During this study it was also found that *B. robustus* is susceptible to diseases and parasites that can affect the species from the egg stage right through to the adult stage. Disease is likely to contribute to the low survivorship of *B. robustus* which has additional pressures from arthropod, avian and mammalian predators (Schori et al. 2019). Current conservation management for this species involves reducing the pressure of mammalian predators, and a complimentary focus on controlling the spread of disease within and between populations could enhance conservation outcomes. This will be an important topic for research if rearing *B. robustus* in the laboratory environment is introduced as a conservation strategy to augment wild or translocated populations.

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Chapter 3 Using radio telemetry to reveal movements of a Nationally Endangered grasshopper in two contrasting habitats with implications for conservation management

3.1. Preface

Selecting appropriate receiving habitat is fundamental to translocation success. During the first translocation of *B. robustus* that took place in 2015 (prior to this thesis), individuals were released into purpose-built receiving habitats measuring 15 m x 15 m, constructed from riverbed gravels, and situated on an historic riverbed that has since evolved into a sparsely vegetated dryland. The grasshoppers were released into their receiving habitat with a 1 m high shade cloth fence constructed around the perimeter of the plot at 1 m from the gravel edge to deter dispersal. Despite the fence remaining in place for > 6 weeks after the translocation, the population was seen to decline by almost a third in the weeks immediately after release (Chapter 4, Figure 4.3). While this may in part be due to death induced by translocation effects such as stress (Teixeira et al. 2007), a portion of the loss is expected to be attributed to dispersal away from the receiving habitat (Le Gouar et al. 2012) which can occur if the habitat does not provide suitable resources such as food, refuges, or oviposition substrate (Chapman and Joern 1990, Le Gouar et al. 2012). Beyond preferring gravel substrate (Bigelow 1967, White 1994) little else is currently known about what habitat features *B. robustus* may require. It is also unclear whether the 15 m x 15 m plots were of substantial size for supporting a population of *B. robustus* because no studies have yet investigated their natural ranging area. In this chapter, radio transmitters are used to track the movements of adult female grasshoppers in an open braided riverbed, and in a modified gravel road habitat where they persist naturally. By comparing movements of grasshoppers in the two contrasting environments, key habitat requirements are identified with important implications for selecting or creating future receiving habitat for translocated *B. robustus*.

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Using radio telemetry to reveal movements of a Nationally Endangered grasshopper in two contrasting habitats with implications for conservation management

Schori, J.C.[1], Steeves, T.E.[1] and Murray, T.J.[2]

1. School of Biological Sciences, University of Canterbury
2. School of Forestry, University of Canterbury

3.2. Abstract

Habitat loss is one of the major drivers of species extinction. However, when pristine habitat has been lost, modified environments may provide a suitable alternative. Whether a species uses modified and natural habitats differently, and the implications this has for conservation management, is often unclear. The Nationally Endangered grasshopper, *Brachaspis robustus,* is a braided river specialist but one of the densest populations occupies an un-used gravel road. Using radio transmitters attached to adult females, movements were compared between the linear road and an open braided river habitat. Dense vegetation was found to be unfavourable indicating that management of vegetation will be important for maintaining habitat quality. No difference in home-range size was found between the two sites indicating that the area of habitat required to support adult females is > 300 m^2. This has applications for managing remaining habitat (e.g. area over which management of weeds and predatory mammals should be implemented), creating artificial habitat, or selecting potential receiving habitats for conservation translocations. Movement was more directional at the road habitat indicating habitat shape modified natural movement patterns. Sheltering behaviour was more common in the natural braided river where substrate was more heterogeneous. The population currently inhabiting the linear gravel road indicated that although expansive habitats with heterogeneous substrate appear to be optimal, these features are not essential for population persistence, and highlights the value of modified habitats for the conservation of threatened insects in the absence of pristine habitat.

Keywords: Orthoptera, *Brachaspis robustus*, New Zealand, radio transmitters, habitat, conservation

3.3. Introduction

Habitat loss is one of the major drivers of species extinction (Fahrig 1997, Sala et al. 2000), but for some threatened species, unnatural, modified environments can provide an alternative habitat when pristine habitat has been lost. These patches of suitable, modified habitat have potential conservation benefits, particularly for threatened invertebrates at low trophic levels (van Nouhuys 2005). Two examples from New Zealand include a population of the At Risk (Recovering) Mahoenui wētāpunga, *Deinacrida mahoenui* (Trewick et al. 2014) which persists within a scrubby reserve dominated by exotic gorse scrub (*Ulex europaeus* L.) (Sherley and Hayes 1993); and the last remaining population of the Nationally Critical ground beetle, *Holcaspis brevicula* (Leschen et al. 2012), which persisted for 80 years in an exotic plantation forest of *Pinus radiata* (Brockerhoff et al. 2005). However, it is often unknown whether the features of an unnatural habitat alter how a habitat is used by a species, and what implications this may have for the protection and ongoing management of threatened species.

The braided rivers of New Zealand provide a unique and dynamic habitat for > 130 species (Caruso 2006, O'Donnell et al. 2016), but in recent decades they have undergone some substantial modifications. Braided rivers feature highly variable flows and multiple channels that weave across wide gravel floodplains (Gray and Harding 2007). In the Mackenzie Basin of the South Island of New Zealand, braided rivers provide habitat for > 30 declining species (O'Donnell et al. 2016). This includes two threatened terrestrial insect species (Trewick et al. 2014) although the threat status of most endemic insects occupying these habitats has not been assessed (Stringer and Hitchmough 2012). The introduction of predatory and herbivorous mammals and exotic weedy species since the arrival of Europeans in the 1800s (Caruso 2006), combined with the installation of Hydro Electricity schemes (Young et al. 2004), has resulted in significant changes to the braided river environment (Caruso 2006, O'Donnell et al. 2016) which include the regulation of river flows and the creation of linear transportation infrastructure for canal and dam construction. Collectively these changes have resulted in the loss of pristine braided river habitat.

Endemic to the Mackenzie Basin is the Nationally Endangered grasshopper, *Brachaspis robustus* Bigelow (Orthoptera: Acrididae) (Stringer and Hitchmough 2012, Trewick et al. 2014). A braided river specialist, *B. robustus* usually inhabits terrestrial rocky habitats associated with flood plains, river braids and terraces (Bigelow 1967, Morris 2002, Trewick et al. 2012). However, one of the largest remaining populations occupies a gravel road (herein referred to as Patersons Terrace) created during construction of a hydro-electric scheme during the 1970s. This road represents a uniform, unnatural, narrow and linear habitat when compared to the natural braided river environment.

Only a few studies have focused on *B. robustus* broadly investigating its taxonomy (Trewick 2001), behaviour (F. Thorsen, unpub. data) and ecology (White 1994, Morris 2002, 2005a, b, Schori et al. 2019). Obtaining detailed information about *B. robustus* is challenging because populations are of low density and patchily distributed, and individuals are both visually and acoustically cryptic (Morris 2005a). Since 1994, the practice for monitoring *B. robustus* involved an observer visually searching all available habitat of interest, with detection depending on the grasshopper moving or jumping in response to the disturbance caused by the observer's presence (White 1994). This is both time-consuming for the observer, and disruptive to the grasshopper. Long-term tracking of individuals is difficult because any identification marks are lost during moulting of juveniles, and the chance of re-finding marked adults is relatively low (White 1994).

In this study, miniaturised radio transmitters are used to overcome some of the challenges associated with studying a highly cryptic insect species. Since their first reported use in 1992 (Riecken and Ries 1992), small radio transmitters have been used to study a number of threatened insects including species of Coleoptera (Rink and Sinsch 2007, Negro et al. 2008), Odonata (Moskowitz and May 2017), and Orthoptera (Gibbs and McIntyre 1997, Watts et al. 2012). Here, we compare differences in movements between *B. robustus* occupying the highly modified habitat to those in an open braided river habitat. We investigate the use of refuges, home range size, and direction of movements. We also provide an evaluation of the use of radio transmitters as a tool to study a highly cryptic, endangered insect in a braided river environment.

3.4. Methods

3.4.1. Site descriptions

This study was conducted in the Mackenzie Basin of the South Island of New Zealand (Figure 3.1). The climate is relatively continental, and temperatures are on average 15 °C in summer and 3 °C in winter, although they often rise above 30 °C and regularly fall below 0 °C in the wintertime (Macara 2016). The region is characteristically dry, receiving an average annual rainfall of 600 mL (Macara 2016). The constant erosion of the alpine and sub alpine mountain ranges which form the perimeter of the basin provide high sediment loads that are conducive to the formation of the braided rivers that traverse the basin.

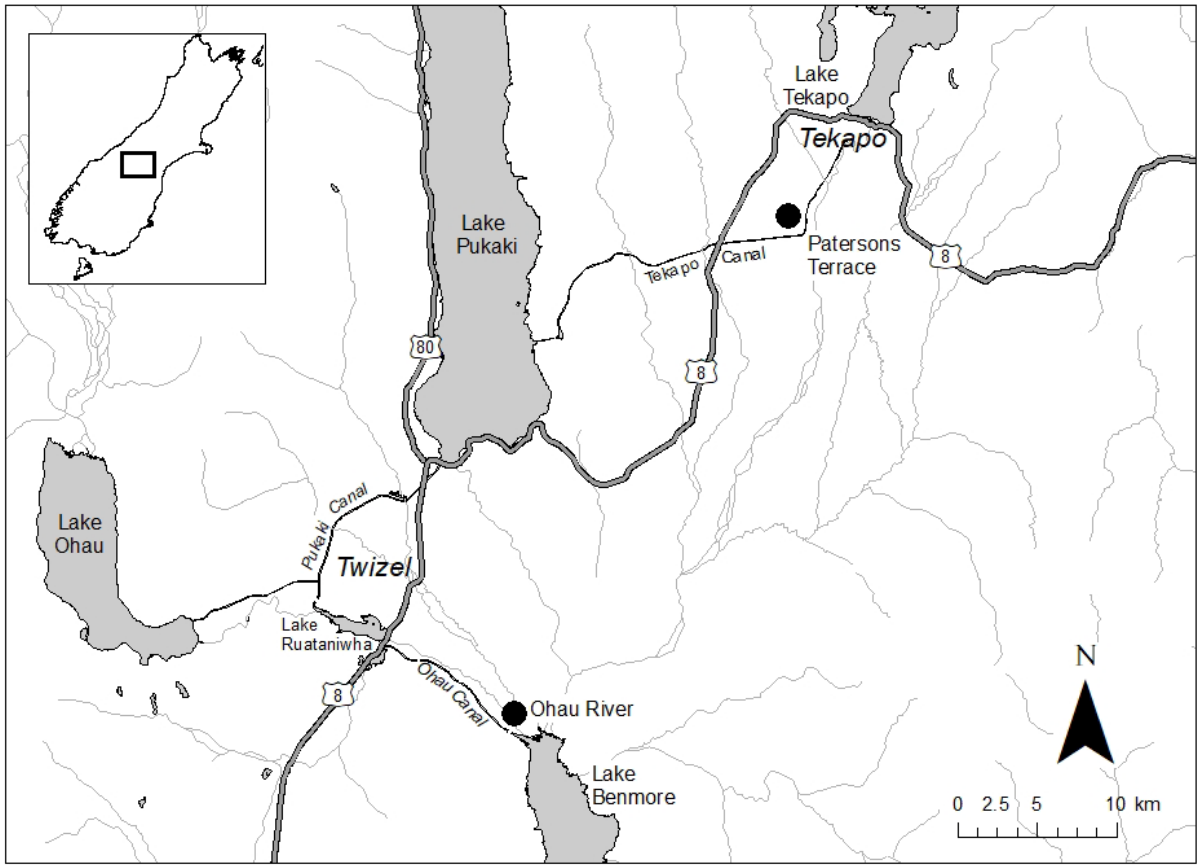

Figure 3.1. The locations of the two study sites; Patersons Terrace and Ōhau River in the Mackenzie Basin (black dots). The Mackenzie Basin is located in the centre of New Zealand's South Island (inset).

The first study site was in the lower reaches of the Ōhau River (alt. ~370 m a.s.l.). The Ōhau River formally drained all of the Lake Ōhau catchment but was dammed for hydro-electric power development in the 1980s, such that over most of its remaining length it has a residual flow of about 1 m^3s^{-1} from spring-fed seepages, with occasional controlled spills of up to 300 m^3s^{-1}. The lower 1.5 km of the river before it flows into Lake Benmore is enhanced with natural flows (mean annual flow 3 m^3s^{-1}) from the Twizel River. The riverbed is comprised of a diverse stony substrate that ranges from sand through to boulder, and spans ~600 m in width. The riverbed under-goes minor disturbances by recreational users and is open to invasive weeds and introduced mammalian predators, but otherwise represents an open braided river. *Brachaspis robustus* have been recorded at this site since the 1980s (DOC, Te Manahuna/Twizel Office).

In comparison, the second site, Patersons Terrace, is a recently constructed unnatural habitat (alt. ~690 m a.s.l.). It is an un-used gravel road that was first established during the construction of the Tekapo Canal in the 1970s (McKay et al. 1978, Trewick et al. 2014). The road substrate is less diverse than Ōhau River, consisting mostly of gravel (small stones < 64 mm diameter) with a few areas of

larger cobbles. The stone cover is dense and has been compacted by historical heavy vehicle use. The available gravel habitat is narrow (~5.4 m wide) and linear. It is boarded by semi-modified grasslands dominated by fescue tussock and exotic pasture grass (Department of Conservation 2004) which are browsed by introduced rabbits (*Oryctolagus cuniculus*) and hares (*Lepus europaeus*) and until recently were occasionally grazed by sheep (*Ovis aries*). A population of *B. robustus* have been recorded at the Patersons Terrace site since 2003 (DOC, Te Manahuna/Twizel Office). It is not reported when or how the population first became established at Patersons Terrace which is somewhat isolated from nearby *B. robustus* populations by hydro-electric canals and steep terraces. Gravel for canal construction was often sourced from the Tekapō River flats (McKay et al. 1978) where *B. robustus* occur naturally, so it is possible they were unintentionally introduced during the 1970s. Alternatively, they may have colonised the road naturally by dispersing south from the Fork Stream.

3.4.2. Field methods

A total of twenty adult female *B. robustus* were tracked using 0.22 g transmitters (Model LB-2X, Holohil Systems Ltd, Canada); ten in the Ōhau River and ten at Patersons Terrace. Tracking began at Ōhau River between 18th of October and 27th November 2017 and at Patersons Terrace several weeks later between the 4th and 6th of December 2017. The staggered deployment times were to account for the later maturation of grasshoppers at the higher altitude site reported by White (1994). Because *B. robustus* is a sexually dimorphic species (males up to 17 mm and females up to 38 mm in body length), it was only feasible to attach transmitters to adult female grasshoppers. To find females at each site, a slow walk (described in Chapter 5) across areas where grasshoppers are known to occur was used. The location where each female was captured was recorded using a GPS (Garmin E-trex 20) before they were transported to the laboratory in small plastic containers with a mesh top stored inside of an insulated bin cooled with two icepacks. In the laboratory, body length (from head to the tip of the abdomen), hind femur length and body weight were measured for each grasshopper.

Before attachment, transmitter aerials were trimmed to ~65 mm in length (approximately half their original length) to ensure minimal inhibition of the grasshoppers' movement through their environment, but still allowing for transmitter detection with the transceiver from up to 6 m away. The transmitter was then attached to the grasshopper using one of two methods. For method one, the transmitter was attached to an aluminium saddle using Selleys® Roof & Gutter translucent silicone sealant, and once set, the saddle was attached to the pronotum of the grasshopper using Selleys® QuickFIX™ No Mess Supa Glue™ (methods adapted from Watts et al. (2012), Figure 3.2A). For method two, the transmitter was super glued to a small square (5 mm by 4 mm) of 2 mm thick polyethylene

foam and then the foam was super glued to the pronotum of the grasshopper (Figure 3.2B). The second method was used more often because the setting time of the super glue (10 seconds) was much faster than the silicon (several hours) and it allowed for easier removal of the transmitter at the end of the study (see below).

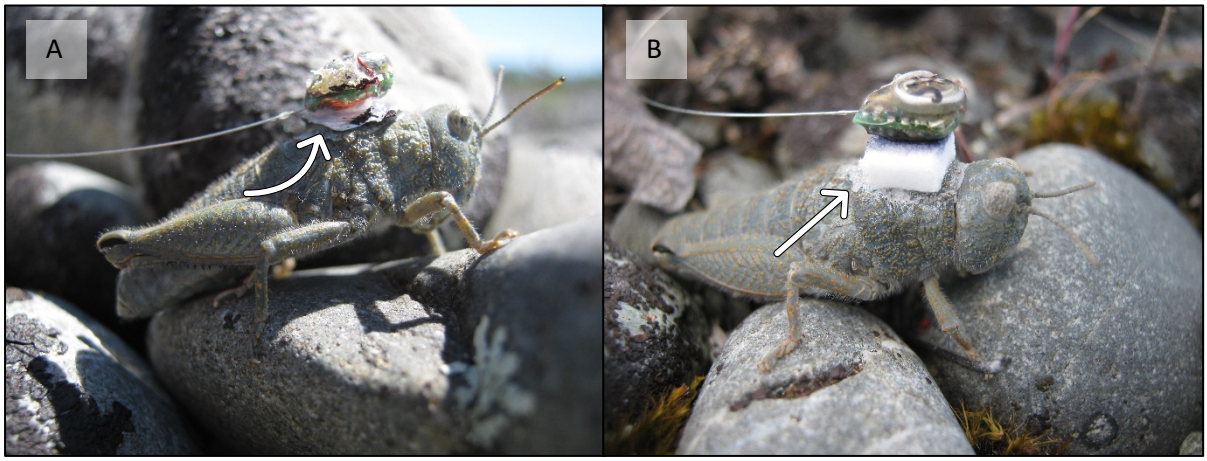

Figure 3.2. Radio transmitters were attached to the pronotum of adult female *B. robustus* using either (A) an aluminium saddle, or (B) a polyethylene saddle.

The grasshoppers were released back to their exact location of capture within 12 hours. Grasshoppers were subsequently located every four hours between 8 am and 8 pm using a transceiver for between one and eleven days, depending on the battery life of the transmitter. Each location event is henceforth termed a 'fix'. At Patersons Terrace, an additional 11 pm search was conducted on a single occasion to determine the night-time behaviour of the six individuals that were being tracked at that time. The location of each fix was recorded using a GPS, and a pink or orange spray-painted rock was placed as close as possible to the grasshopper without disturbing it to provide a reference point from which to begin the next search. Ground surface temperature in the shade, air temperature, and relative humidity (at 1 m above ground) were measured using a Kestrel® 3000 Weather Meter, and observations on wind (categories; none, light, strong) and cloud cover (categories; clear, high cloud, patchy cloud, overcast, precipitation) were recorded at the start of each search event. Grasshopper activity (categories; eating, moving, mating, basking/still) and a description of its immediate surroundings (including; "exposed" to sunlight, "shaded" or "partially shaded" from sunlight, "sheltered" from the wind) were recorded for each fix. Two HOBO® H21-002 micro station loggers were used to record air temperature (~20 cm above ground surface) and relative humidity (HOBO® S-THB-M008 smart sensor), and temperatures on, and ~3 cm below the ground surface (HOBO® S-TMB-M006 smart sensors) at one minute intervals over the monitoring period. One logger was set up on the Patersons Terrace road, and one logger was set up 8.5 km up-stream of the Ōhau

River site (to avoid interference by the public). On four of the scheduled occasions, monitoring was not conducted because electrical storms compromised observer safety when using the telemetry aerial. If the transmitter could not be detected using the transceiver (e.g. indicating transmitter battery failure) visual searches for the grasshopper were conducted around the last recorded location for up to 90 minutes. If found, inactive transmitters were removed; those attached with foam were peeled off by hand in the field, while those attached with aluminium saddle were returned to the laboratory to cut through the silicon layer using a scalpel. In most cases all traces of the foam and glue could be removed. The grasshopper was then marked with a unique ID (methods adapted from Buchweitz and Walter (1992)) using a non-toxic paint pen (Edding® 780 gloss paint marker) before release so they could be identified if observed at a later date.

3.4.3. Data analyses

All statistical analyses were conducted in *R* (R Development Core Team 2011) except where otherwise stated. A Wilcox t-test (1000 permutations) was used to test if ground temperature differed at exposed, shaded and partially shaded grasshopper fixes. Fisher's Exact test was used to determine if grasshopper position in the habitat was random with respect to cloud and wind conditions. This was assessed separately for the Ōhau River and Patersons Terrace sites.

Distance travelled (the sum of the distances between all consecutive fixes) and relocation distance (the straight-line distance between the first and last fix over a defined time period), were measured in ArcMap. Home range analyses were conducted using *Ranges 9* software (Anatrack Ltd). Home ranges were calculated using minimum convex polygons with arithmetic mean centres, 100 % cores and 3 m tracking resolution. Only individuals tracked for 3 or more days, with 5 or more fixes, were included in home range analysis (Ōhau River n = 8, Patersons Terrace n = 7). Kendall's rank correlation was used to check for correlation between home range size and the number of fixes, and home range size and the number of tracking days. Because a strong correlation was found, only home range estimates generated for individuals that were tracked for between 3 and 7 days were used to compare the home range sizes of the two populations (Ōhau River n = 7, Patersons Terrace n = 4), using Wilcox t-test (1000 permutations). Estimated home range for all tracked grasshoppers was modelled against the study population (Ōhau River v. Patersons Terrace) and number of tracking days using a linear model with the *lme4* package (Bates et al. 2015). Model fit was assessed by inspecting residuals and best fit was achieved with a log transformation of the dependant variable (home range size).

To assess how grasshopper movement changed over time with transmitter attachment, we measured relocation distance (m) over 24-hour intervals since time of release, and modelled distance using a linear mixed effect model with the *lme4* package (Bates et al. 2015). Average daily air temperature (°C), ratio of transmitter weight to the individual's body weight, and study population (Ōhau River v. Patersons Terrace) were incorporated as co-variates, and individual ID was specified as a random effect. Model fit was assessed by inspecting residuals and best fit was achieved with a square root transformation of the dependant variable (relocation distance). Model selection was conducted by testing the significance of temperature, location and day variables using ANOVA, and selecting for a model with low AIC. The absolute turning angles (the angle between north and the direction of travel) and relative turning angles (the angle between direction of travel and the previous direction of travel) between fixes taken at 24-hour intervals since time of release were calculated using the *adehabitatLT* package (Calenge 2006). A Rayleigh test (Wilkie 1983) was used to test for significant mean directionality of turning angles for each population. A Watson two-sample test was used to compare the circular distribution of absolute turning angles between the two populations.

3.5. Results

3.5.1. Grasshopper activity and movement

Grasshoppers were most frequently found in exposed (Ōhau River = 68.8 %, Patersons Terrace road = 92.7 %) rather than shaded or sheltered positions (Ōhau River = 31.3 %, Patersons Terrace = 7.3 %), and on rocky substrates (Ōhau River = 90.6 %, Patersons Terrace = 70.8 %) rather than vegetation (Ōhau River = 9.4 %, Patersons Terrace = 22.5 %) or soil (Ōhau River = 0 %, Patersons Terrace = 6.7 %). The vegetation types that grasshoppers were found on were grass, mat daisies (*Raoulia australis*) or hawkweed (*Hieracium* spp. and *Pilosella* spp.), and shade was provided either next to a large rock or underneath leafy vegetation (briar rose *Rosa rubiginosa* or Californian poppies *Eschscholzia californica*). Overall, grasshoppers were found basking/still 78.8 % of the time, mating 13.4 % of the time, moving (walking, jumping) 6.9 % of the time and eating 1 % of the time. Nine of the twenty females were observed copulating with a male during the study period (Ōhau River, *n* = 4, Patersons Terrace, *n* = 5) and of those, seven were observed copulating at least twice (Ōhau River, mean = 2.25 copulation events per copulated female; Patersons Terrace, mean = 2.8). The most copulation events observed for a single female during the study was five at Ōhau River (Patersons Terrace, max. = 4). Although no females were observed ovipositing, on two occasions a female was found with dust covering her abdomen as would be expected after an oviposition event.

A significant difference in ground temperature was observed (Welch's t-test; t = -3.50, d.f. = 45.9, p = 0.001, permutation test; min = -2.78, max = 3.98, test statistic = -3.50) for grasshoppers located in shaded/partially shaded (mean = 29.4 °C, range = 17.3 °C to 39.1 °C) compared to exposed (mean = 25.8 °C, range = 13.7 °C to 39.1 °C) positions. At Patersons Terrace, there was no evidence that grasshopper positions (exposed, sheltered, shaded and partially shaded) were correlated to cloud cover or wind conditions (Fisher's exact test, p = 0.07, p = 0.4 respectively, Figure 3.3) however position was significantly correlated to cloud cover and wind at the Ōhau River, (Fisher's Exact test, p = 0.02 and p = 0.04 respectively. Figure 3.3).

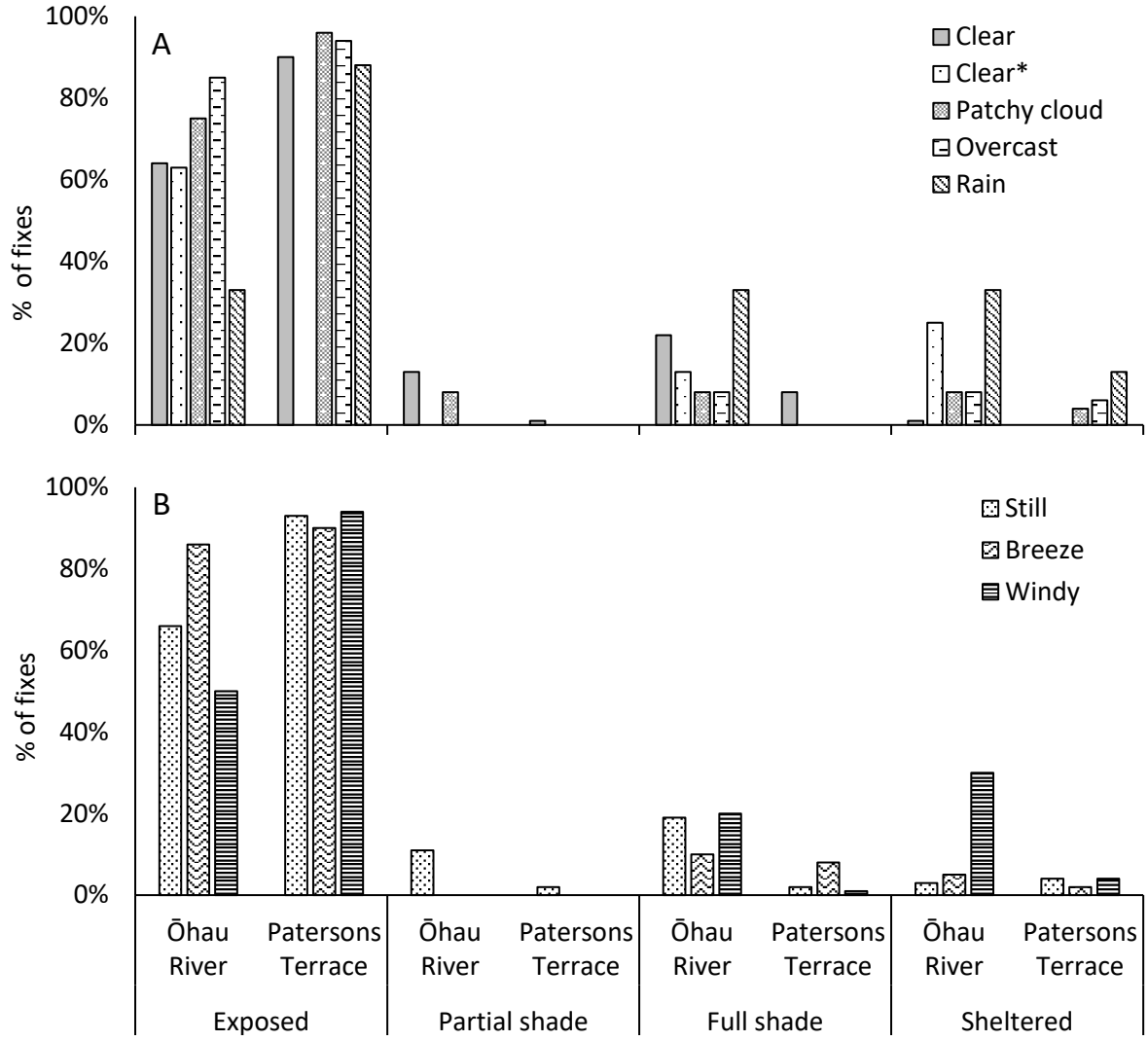

Figure 3.3. The frequency (% of fixes) of which grasshoppers were found in exposed, partial shade, full shade, or sheltered locations under the range of cloud (A), and wind conditions (B), at the two monitoring sites; Ōhau River (open riverbed) and Patersons Terrace (unnatural gravel road). *In several instances at the Ōhau site, monitoring occurred on a clear evening however the sun was behind a large hill near to the site. In these cases, because there was no direct sunlight on the monitoring location it was placed in the separate category, clear*.

On occasion, grasshoppers at the Patersons Terrace site were observed to move off the gravel road and onto the road margin where the surface was a mixture of exposed soil, stones, hawkweed and short, sparse grasses. They were never detected more than 7 m from the edge of the gravel road and were never observed in the taller, denser grasses that were present beyond the road margin. On the single occasion that grasshoppers were tracked at 11 pm, all six females were observed to be on the surface of the stones on the road and were found within 30 cm of their previous location recorded

at 8 pm. They did not appear to be disturbed by the flashlights and remained unresponsive when approached and photographed. The ground temperature at the time was 17 °C.

The furthest distance an individual was recorded from the initial release point was 65 m after 10 days at Patersons Terrace (Figure 3.4, individual P30a). The maximum cumulative distance travelled between all consecutive fixes was 148 m at Patersons Terrace over 11 days (Figure 3.4, individual P27). The maximum relocation distance of an individual in a single night (8 pm – 8 am) was 9 m (individual P18), however the combined mean from both sites was < 2 m (s.d. 1.5 m). There was a significant positive correlation between home range size and the number of tracking days (Kendall's rank correlation; $\tau = 0.55$, $p = 0.001$) and between home range size and the number of fixes (Kendall's rank correlation; $\tau = 0.57$, $p < 0.001$). For individuals tracked for between 3 and 7 days, average home range size was 266.0 m^2 (s.d. 285.2 m^2) at Ōhau River and 296.5 m^2 (s.d. 122.2 m^2) at Patersons Terrace and did not differ significantly between the two locations (permutation test, min = -2.54, max = 2.80, test statistic = -0.25; Figure 3.5). The maximum estimated home range size of any individual was 877.9 m^2 at Ōhau River (Figure 3.5, Individual O26, tracked for 6 days), and 628.1 m^2 at Patersons Terrace (Figure 3.5, Individual P27, tracked for 11 days). Home range size increased on average by 19 % for every day of tracking (s.d. = 6 %, $p = 0.003$), and did not significantly differ between the two locations ($p = 0.46$, Figure 3.6).

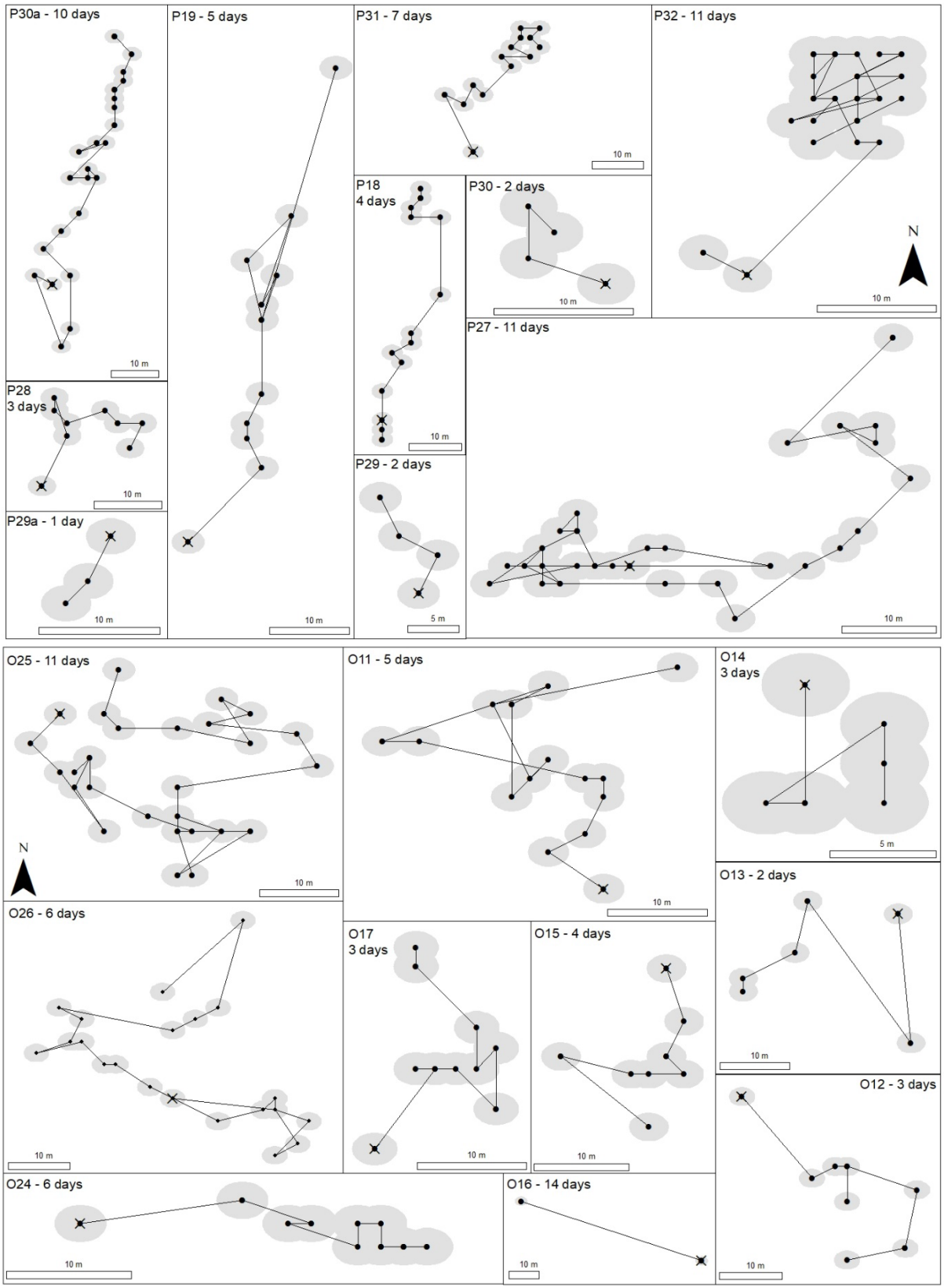

Figure 3.4. The tracked movements of 20 adult female *B. robustus* grasshoppers at Patersons Terrace road (individuals P18, P19, P27-32, top) and Ōhau River (individuals O11-17, O24-2,6 bottom). Shaded areas represent known GPS uncertainty (±3 m). For each individual 'X' represents the release point. The number of tracking days for each individual is indicated in figure.

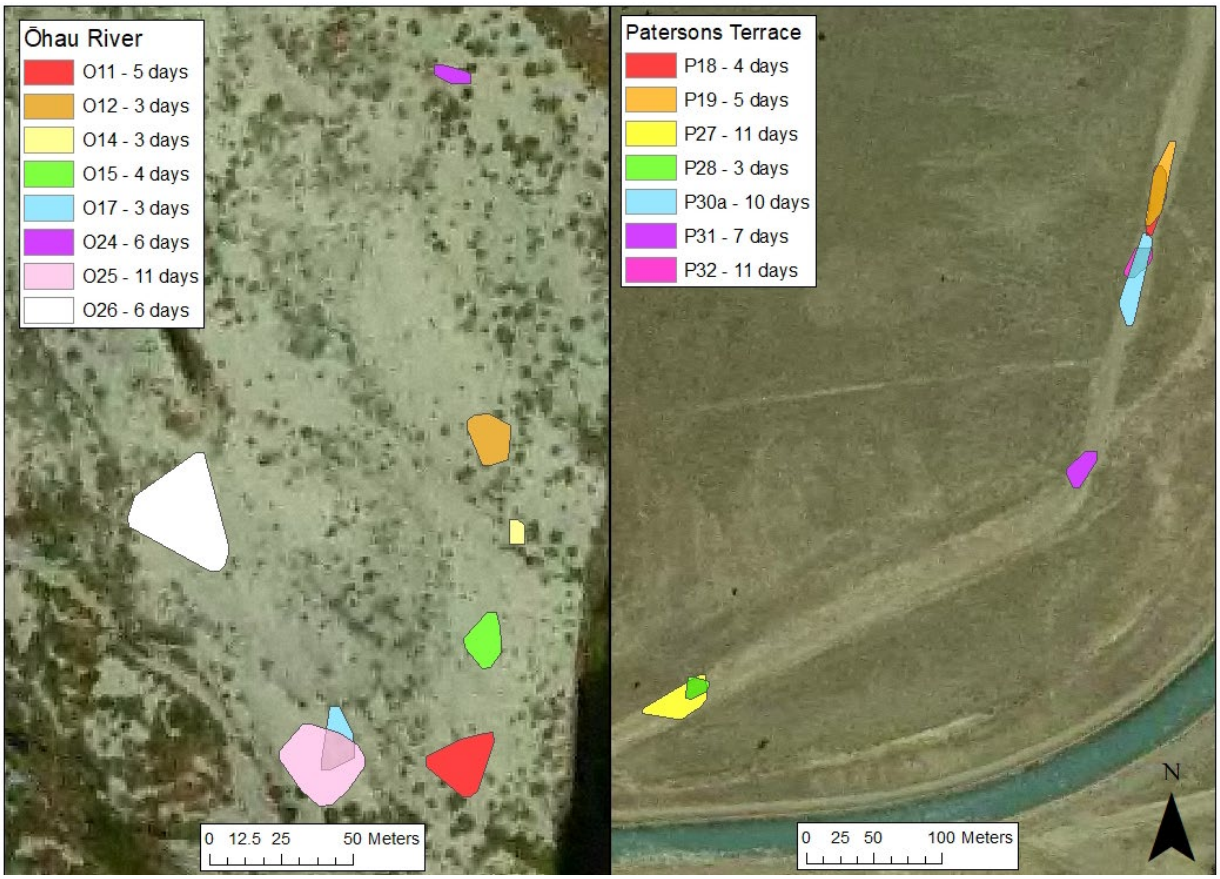

Figure 3.5. The home ranges (calculated using MCPs, 100% cores) of 15 adult female *B. robustus* tracked for 3 or more days using radio transmitters at Ōhau River (left) and Patersons Terrace (right) in 2017. The number of days over which home range was calculated for each individual is indicated in the legend.

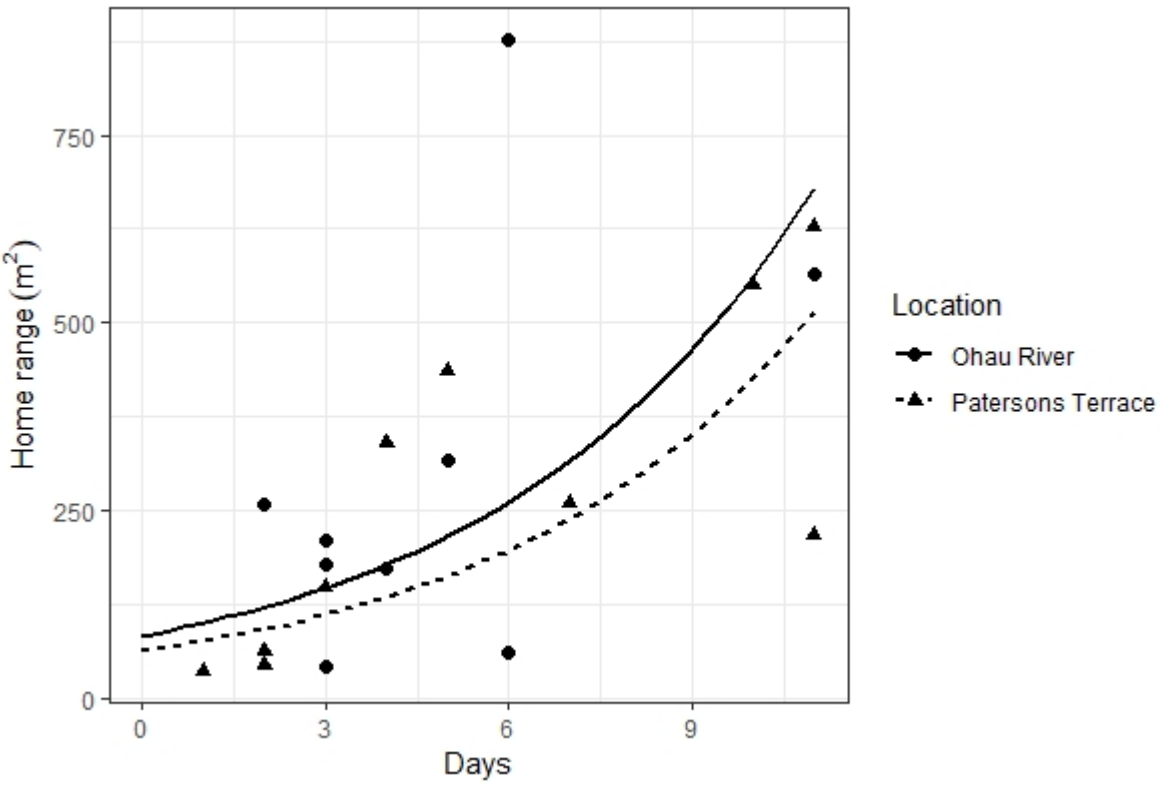

Figure 3.6. The relationship between the number of days that an adult female *B. robustus* was tracked for and the estimated home range size (m^2) at Ōhau River and Patersons Terrace.

Daily relocation distances were found to decline as days since release increased, however evidence was not strong given large standard errors (-0.08 ± 0.04 SE, d.f. = 75, p = 0.03, Figure 3.7). Daily relocation distance decreased as the proportional weight of the transmitter to the individual's body weight increased (-10.25 ± 3.00 SE, d.f. = 75, p = 0.001, Figure 3.7), and individuals at Patersons Terrace relocated less linear distance in a 24-hour period than those at Ōhau River (-0.61 ± 0.21 SE, d.f. = 75, p = 0.006, Figure 3.7).

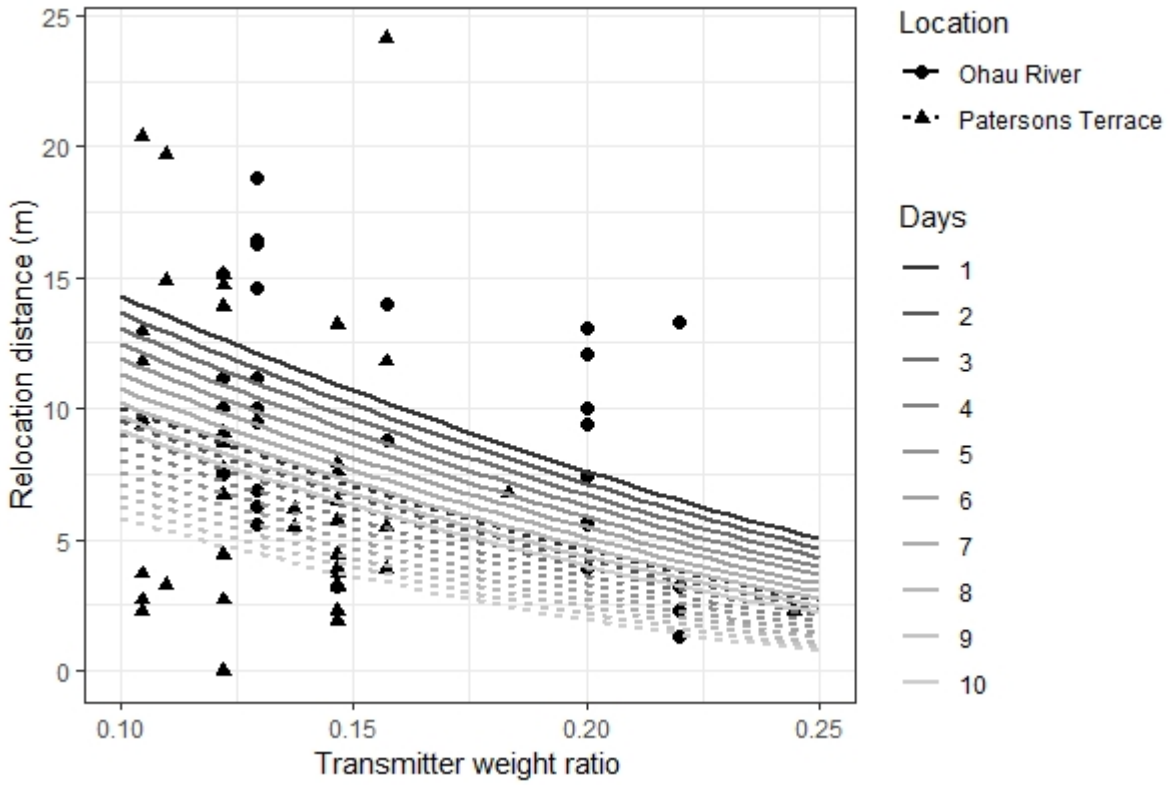

Figure 3.7. The relationship between the linear distance (m) that an adult female *B. robustus* grasshopper relocated within a 24-hour period, the number of days since tracking began and the transmitter weight to grasshopper body weight ratio at Ōhau River and Patersons Terrace.

The distribution of absolute turning angles (the direction of travel relative to north) at 24-hour intervals differed significantly between Ōhau River and Patersons Terrace (Watson two-sample test; t stat = 0.25, critical value = 0.19 at p = 0.05), and showed significant directionality at Patersons Terrace (mean = 25.01 °, t stat = 0.40, p < 0.001) but not at Ōhau River (mean = 88.28 °, t stat = 0.08, p = 0.83, Figure 3.8). The distribution of relative turning angles did not differ between the two populations (Watsons two-sample test; t stat = 0.06, critical value = 0.19 at p = 0.05) and did not show significant directionality at either site (Ōhau River, mean = 112.05 °, t stat = 0.40, p = 0.95; Patersons Terrace, mean = -70.04 °, t stat = 0.19, p = 0.27).

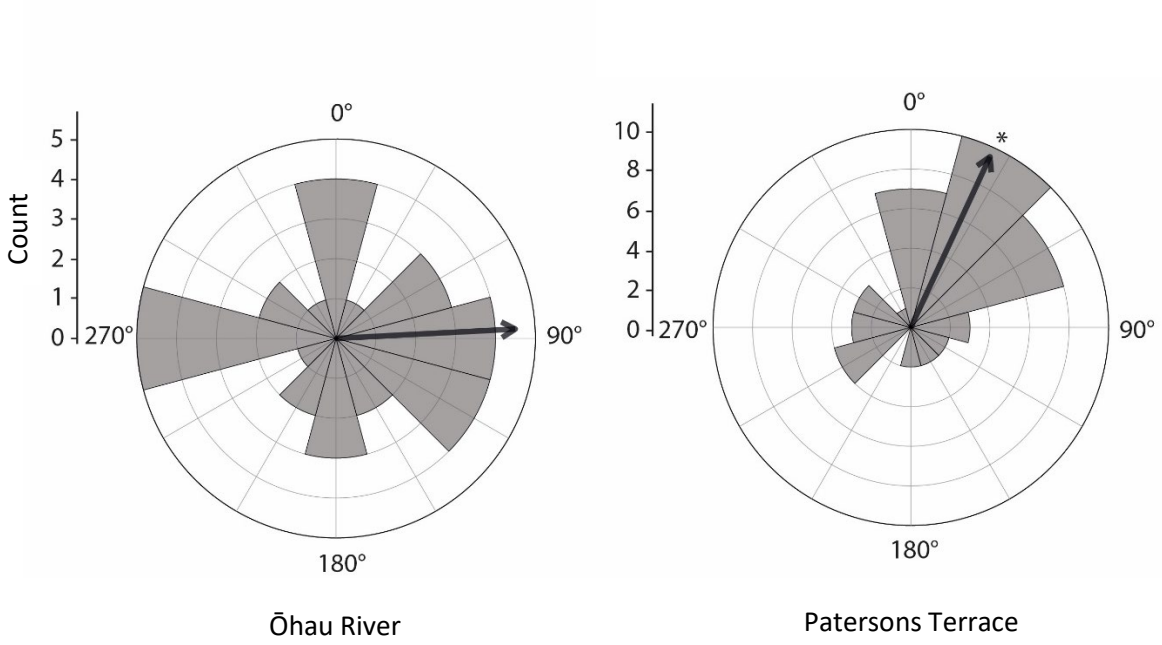

Figure 3.8. The distribution of absolute turning angles (the direction of travel relative to north) for *B. robustus* at the Ōhau River and Patersons Terrace in each 24-hour interval of tracking. Arrow indicates mean direction of travel for each site; * indicates significance at $p < 0.001$.

3.5.2. Transmitter use and performance

The mean weight of adult female *B. robustus* was 1.5 g (s.d. = 0.3 g). The 0.22 g transmitters weighed between 10 % and 24 % of their body weight (mean = 15 %, s.d. = 4 %). The transmitter battery life ranged from 8 to 270 hours (mean 143 hours). Half of the transmitters had been in storage for approximately 12 months which reduced the runtime in the field to ~3.3 days compared to ~7.8 days for transmitters that were newly sourced. For the Ōhau River females, tracking ended in five instances because the transmitter battery died, in one instance because the grasshopper was found dead and in four instances because there was no sign of the transmitter nor the grasshopper following substantial searching of the area surrounding the last sighting. Individual O16 was released at 8 pm but the battery had died when she was found the following morning. She was found again 14 days later and recognised by the unique ID painted on her back (O16, Figure 3.4). This was the only female re-sighted following the removal of the transmitter. At Patersons Terrace, tracking ended in five instances because the transmitter battery died, in two instances because the grasshopper was found dead, and in three instances only the transmitter was recovered. In the three instances where only the transmitter was recovered, two were found at 4 pm after a live sighting of the female at noon, and one at 8 am after a live sighting of the female at 8 pm the previous evening. Both transmitters

found at 4 pm had twisted and bent aerial wires, while that found at 8 am was intact, however the foam used to attach it to the grasshopper had been torn.

3.6. Discussion

3.6.1. Grasshopper activity and movement

During the day, grasshoppers were more frequently found in exposed locations than in shaded or sheltered locations. This is expected given the importance of basking for grasshopper development and reproduction (Forsman 2001, O'Neill and Rolston 2007). Shade and shelter seeking behaviour has previously been observed for *B. robustus* during the hottest part of the day and during strong winds at Snowy River (F. Thorsen, 2010, unpublished data), an alluvial fan with characteristics of a braided river. The present study found grasshoppers in shaded locations at Ōhau River more frequently than at Patersons Terrace. However, Patersons Terrace offers fewer shaded refuges than on the Ōhau River. The stones making up the Patersons Terrace substrate are smaller and more densely packed, compared to open braided rivers where heterogeneous substrate provides interstitial spaces and large boulders provide patches of shade. Further, the vegetation at Patersons Terrace is mostly of lower stature (e.g. mat daises and hawkweed, with the exception of the grassy vegetation which makes up the habitat border) compared to Ōhau River where the vegetation, although sparse, is more diverse and taller plants (e.g. briar rose and Californian poppies) provide shade. Limited access to natural refuges, as appears to be the situation at Patersons Terrace, has several potential implications for grasshopper survival including suboptimal thermoregulation if shade is not available when required, and increased vulnerability to predation and poor weather such as snow if appropriate refuges are not available. It is possible that the human-made habitat at Patersons Terrace currently supports a smaller population than the habitat area could potentially support because a lack of refuges results in regular small losses of individuals through predation and weather events. However, major disturbance events like flooding that remove a significant proportions of *B. robustus* from populations in natural braided rivers (Appendix B) do not occur at Patersons Terrace. This could explain why the population persists at this site despite an apparent low availability of natural refuges.

Observations during the night-time monitor and relatively small relocation distances between the 8 pm and 8 am monitors provide some evidence that this species is not active at night. During the study period, sunrise occurred between 6.30 am and 5.50 am and sunset between 8.15 pm and 9.25 pm, meaning there was sunlight present before the first, and after the last daily fixes. Although night-time temperatures dropped below 14 °C on most nights, evening and morning temperatures were

often warmer than 14 °C. It is likely that a combination of warm temperatures and daylight occurring before the first, and after the last monitor explain the activity seen between 8 pm and 8 am. That all six individuals were observed on the surface of the substrate at 11 pm indicates that this species does not seek refuge at night, a behaviour which makes this species vulnerable to introduced nocturnal predators such as cats (*Felis catus*), ferrets (*Mustela furo*) and hedgehogs (*Erinaceus europaeus*) (Sanders and Maloney 2002).

Home range size did not differ significantly between the linear, modified habitat (Patersons Terrace) and the open braided river habitat (Ōhau River) despite very different habitat shapes. At Patersons Terrace, the suitable habitat available is very long and narrow (~5.4 m wide) with a natural border of tall grassy vegetation along either side. In comparison, the available habitat at Ōhau River is more expansive (~600 m wide) with the natural barriers such as dense vegetation and water bodies spaced much further apart. Distance to resources (such as to food and to areas suitable for oviposition) were not measured in the current study but commonly influence home range size of mammals and birds (Marable et al. 2012, Corriale et al. 2013, McClintic et al. 2014). A lack of difference in home range size could indicate that both sites had adequate availability of resources for female grasshoppers. However, a more likely explanation is that the time over which grasshoppers were tracked for during this study was too short to evaluate a difference. Estimated home ranges were small, and are likely to be underestimates of true ranging area (Kissling et al. 2014) because of short tracking time and possible energetic costs associated with transmitter attachment (discussed in section 3.6.2). However, these results provide a first estimate of the minimum habitat area required for adult female *B. robustus.* We observed home ranges overlapping on several occasions throughout the study period, and no antagonistic female-female behaviour has been observed to date, indicating that it is unlikely that females defend territories. However, the extent to which home ranges can overlap, or the density at which females can occur is not known.

The movement patterns of adult female *B. robustus* at the open braided river site and the linear road site tended to reflect habitat shape and the linear habitat shape appeared to restrict natural movement. At Patersons Terrace, tracked grasshoppers generally moved along the length of the road (e.g. P18, P19, P30a), and had a significant mean direction of travel in the NNE direction. Access to the site was through a gate to the SW of the road, and although effort was made to not walk along the road itself except to locate a specific grasshopper, it is possible that regular observer approach from the SW direction caused grasshoppers to all travel in the NE direction. There were no observations of movement off the gravel road and into the adjacent taller vegetation in this study despite *B. robustus* having occasionally been observed in the small gravel patches interspersed in the longer vegetation (F. Van Eyndhoven, pers. comms.). The movements of Ōhau River females appeared

to be less directional, and individuals tracked for longer periods of time often returned to an area they had visited several days earlier (e.g. O11, O25). Although access to this site was always from the same location, observer approach to a grasshopper did not consistently occur from the same direction at Ōhau River. Because fixes were taken at 4-hour intervals, there is no knowledge of where or how individuals moved in the interim. It is possible that grasshoppers at Patersons Terrace moved further between fixes than Ōhau River grasshoppers because they more frequently encountered the unsuitable edge habitat forcing them to continue searching for optimal habitat or resources. Therefore, a narrow habitat such as Patersons Terrace could increase energetic costs to *B. robustus* grasshoppers. However, it may also facilitate mate finding by increasing the rate of chance encounters. Compared to Ōhau River, more grasshoppers were observed copulating at Patersons Terrace, and grasshoppers also copulated more frequently. These findings provide support for the latter hypothesis, but do not provide strong evidence because of confounding factors such as tracking time, transmitter attachment and differences in time of year that tracking took place. Expansive habitats are recommended for *B. robustus* over linear habitats because they support natural movement patterns, but linear habitats like the road habitat provide a suitable alternative.

Little is known about how males utilise their environment or what their home range size and dispersal patterns are. However, the rarity of *B. robustus* females in the environment suggests *B. robustus* has a prolonged searching polygyny mating system, where males of this species are likely to search for receptive females which are distributed unpredictably throughout the environment (Wickman and Rutowski 1999). This theory is further supported by small male body size which makes the cost of moving less than for the larger and heavier females, both in terms of energetic expense and risk of predation (Wickman and Rutowski 1999). If prolonged searching polygyny is the dominant mating system for this species, it would mean males are likely to roam further than females because they must search for a receptive mate.

3.6.2. Transmitter use

Transmitters proved to be an effective tool for locating and observing adult female *B. robustus* over multiple days. The short battery life of some of the transmitters that had been in storage, and the loss of individuals throughout the study limited the length of time over which some individuals could be tracked. Overall the method was much less disruptive, and much more time efficient compared to previously used visual searching methods (White 1994). The weight of the transmitters were always less than 30 % of grasshopper body weight which falls into the normal range for insect studies (Kissling et al. 2014) but is much higher than the 5 % “rule” often applied to flying vertebrates (Aldridge and

Brigham 1988, Barron et al. 2010), and the ≤ 10 % recommended for lizards (Knapp and Abarca 2009). The transmitters themselves did not appear to inhibit adult female *B. robustus* from performing regular activities such as mating, feeding and walking or jumping, except when moving through vegetation. However, *B. robustus* appear awkward when manoeuvring through vegetation even without a transmitter attached (pers. obs.).

There is likely to be an energetic cost associated with carrying a transmitter, particularly when it is attached for an extended period of time (Kissling et al. 2014). There was a gradual decline in *B. robustus* daily relocation distances over time, and smaller relocation distances for grasshoppers for which the transmitter weighed a higher percentage of their bodyweight. These two observations could be indicative of a cumulative energetic cost, or a constant energetic cost respectively. However, the trends could equally be explained by an aging effect. The age and the health of the grasshopper was not known at the time the transmitter was attached, however around 87 % of adult *B. robustus* females will reach the end of their natural life in December (Chapter 5) suggesting the females tracked in the later stages of this study were approaching the end of their natural life. Alternatively, GPS error could have substantially inflated or deflated the distances travelled given it was large (± 3 m) relative to the distances travelled by grasshoppers in this study. Finally, the observations could be due to chance alone, in that we only encountered the grasshoppers at four locations within a 24-hour period and have no knowledge of where they travelled in the interim. Overall the current study provides some insights into the energetic cost of transmitter attachment, but to fully understand the cost would require a study specifically designed to investigate this question.

The frequency at which tracking ended due to a grasshopper death or loss was higher in this study (55 %) than reported in similar studies on invertebrates (e.g. 20 %, wētā, *n* = 5, Gibbs and McIntyre (1997); 27 %, stag beetles, *n* = 56, Rink and Sinsch (2007); 17 %, wētā, *n* = 42, Watts et al. (2012); 11 %, stag beetles, *n* = 55, Tini et al. (2017); but *not* 100 %, dragonflies, *n* = 7, Moskowitz and May (2017)). However, some previous studies have been on nocturnal species (e.g. wētā; Watts et al. (2012)) or on species present in wildlife sanctuaries (e.g. wētā; Gibbs and McIntyre (1997), Watts et al. (2012)). In three instances in this study individuals were found dead with the transmitter still intact. In all three cases, the individuals showed no external damage to the body making cause of death difficult to determine. One explanation might be that the glue used to attach transmitters could have been toxic to *B. robustus*. However, if that were the case it would be expected that a higher percentage of the individuals in the study would be affected (Boiteau et al. 2009). It also seems unlikely given the same glue has previously been used to attach transmitters to wētā (Watts et al. 2012). Another explanation might be stress-induced death. Because grasshoppers were taken to the laboratory for transmitter attachment, all individuals will have undergone stress similar to that experienced during

a translocation. Stress induced by handling of vertebrates is known to have side effects such as suppressed immune function and starvation (Teixeira et al. 2007, Dickens et al. 2010). Alternatively, females may have simply died from natural causes such as old age and/or poor health (as noted above) which was not known when transmitters were attached. All three that died did so in the first week of December, and all three had high transmitter weight to body weight ratios (24 %, 22 % and 18 %) indicating they were the lightest grasshoppers included in this study. We recommend future studies aim for transmitters to weigh ≤ 15 % of *B. robustus* body weight to reduce energetic cost and maximise longevity of the grasshopper during the study, particularly while tools to assess health and age are not yet available.

All three instances where grasshoppers were lost and only the transmitter was recovered occurred at the Patersons Terrace site. It is suspected that these individuals were killed by predators. Patersons Terrace is known to have avian, reptilian and mammalian predators present (Pierce 1987, White 1994, Norbury et al. 2009) which are likely to prey on grasshoppers opportunistically (White 1994, Schori et al. 2019). Local predators that are active between 12 pm and 4 pm include stoats (*Mustela erminea*), birds (Sanders and Maloney 2002), cats (Pierce 1987), and skinks. Diurnal predators tend to be visual hunters, and it is possible the glinting transmitter aerial could attract their attention. For nocturnal predators that are olfactory hunters (e.g. hedgehogs), transmitter attachment probably has a negligible impact on grasshopper vulnerability because, as reported above, *B. robustus* do not appear to seek refuges after dark. Whether transmitter attachment increases vulnerability of an insect to a predation event has drawn mixed conclusions in the literature (Le Gouar et al. 2015, Moskowitz and May 2017) and further research is needed to draw conclusions about whether transmitter attachment inflated base-line predation rates of *B. robustus*.

3.6.3. Conservation implications

Comparing movements of *B. robustus* in a human-made linear habitat and an open riverbed habitat provided several key insights into the habitat requirements of this grasshopper with implications for conservation management. First, home range size was comparable between a narrow linear habitat and an expansive open habitat. The home range size observed in this study gives an indication of the minimum area of habitat required to support adult female *B. robustus*, which is a critical consideration when managing remaining habitat (e.g. weed and predator control areas), creating artificial habitat, or selecting potential receiving habitats for conservation translocations. Because the home ranges reported in this study are likely to be underestimates of true home range size, we recommend that any future conservation translocations for this species utilise habitats larger than the home ranges

reported here. Second, movement was more directional at the human-made linear habitat indicating habitat shape modified natural movement patterns. Sheltering behaviour was more common in the natural braided river where substrate was more heterogeneous. Although expansive habitats with heterogeneous substrate appear to be optimal, these features are not essential for population persistence as evidenced by the population currently inhabiting the linear gravel road. Third, grasshoppers were never seen to move through the dense vegetative border on Patersons Terrace, indicating that the grass verges and dense vegetation are unfavourable habitat for *B. robustus*. This is of potential concern because both Patersons Terrace and the Ōhau River have recently become low disturbance environments which facilitates vegetation growth. The Ōhau River no longer has large natural flooding events because of an up-stream hydro-electric dam, and Patersons Terrace has recently become managed by the Department of Conservation which have removed grazing sheep and have goals to eradicate browsing lagomorphs from the area. Vegetation management will be important for maintaining habitat quality including connectivity within populations of *B. robustus* at Patersons Terrace and in other flow-regulated braided rivers.

Despite a narrow, linear habitat shape and an apparent lack of natural refuges from weather extremes and predators, the human-made gravel road habitat provides habitat of adequate quality to support one of the densest remaining populations of the Nationally Endangered grasshopper *B. robustus*. Further, even small patches of modified habitats have potential to provide conservation benefits for threatened insects when pristine habitat has been lost, as observed by the home range size of female *B. robustus* tracked in this study. This study has highlighted the value of modified habitats for the conservation of threatened insects in the absence of pristine habitat.

3.7. References

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Chapter 4 Evidence that reducing mammalian predators is beneficial for threatened and declining New Zealand grasshoppers

4.1. Preface

Another key consideration of the translocation receiving habitat is that threats to species persistence are not present. Several potential threats to *B. robustus* were identified in the Introduction of this thesis, one being introduced mammalian predators. If mammalian predators pose a substantial threat to *B. robustus,* then translocation success will rely on their populations being suppressed in the receiving habitat to levels at which *B. robustus* populations can grow. Currently it is unknown which mammals should be targeted, and the extent to which populations need to be supressed to alleviate the threat to *B. robustus*. Here, the outcome of the translocation that experimentally released grasshoppers into a predator reduced area and a non-predator reduced area in 2015 is evaluated. This is compared to long-term trends of three populations of another declining dryland grasshopper species, *Sigaus minutus*, that are present in areas where mammalian predators are controlled at different levels of intensity. The results presented in this chapter have direct applications for improving future translocation success of *B. robustus* by informing the appropriate level of mammalian predator control to implement within the receiving habitat to maximise success.

4.2. Statement of contribution

This chapter has been published as:

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The PDF of the published manuscript can be found here: DOI 10.1080/03014223.2018.1523201. The work presented in this chapter is the same* in content to the published research article. As the first author of this manuscript, I designed and executed the data analyses, created the figures and wrote the manuscript. The manuscript was developed with feedback from co-authors Dr. Tara Murray, Assoc. Prof. Tammy Steeves, Dr. Richard Maloney, and two anonymous reviewers. Monitoring of translocated *B. robustus* grasshoppers was conducted by Tara Murray with support from Te Manahuna/Twizel Department of Conservation staff in 2014 and early 2015, and by me from late 2015 onwards. Data for *S. minutus* was generously provided by Te Manahuna/Twizel Department of

Conservation (DOC). Tracking tunnel data was collected by Te Manahuna/Twizel DOC staff in March 2015, and by me from December 2015 onwards.

4.3. Erratum

*The published manuscript incorrectly states that a *Poisson mixed effects model* was used during data analyses. The version of the manuscript presented in this chapter correctly states that a *generalised linear model with a Poisson distribution* was used.

Evidence that reducing mammalian predators is beneficial for threatened and declining New Zealand grasshoppers

Schori, J.C.[1], Maloney, R.F.[2], Steeves, T.E.[1], Murray, T.J.[3]

1. School of Biological Sciences, University of Canterbury, Christchurch, New Zealand
2. Biodiversity Group, Department of Conservation, Christchurch, New Zealand
3. School of Forestry, University of Canterbury, Christchurch, New Zealand

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4.4. Abstract

We evaluate evidence that reducing mammalian predators benefits threatened and declining grasshoppers in the Mackenzie Basin, New Zealand. Long-term population trends of *Sigaus minutus* are investigated under three control regimes; high intensity, indirect control through prey reduction and no control. We then test whether predator control benefits conservation management of *Brachaspis robustus* by translocating wild-caught individuals to areas of moderate versus no predator control. A significant positive trend of *S. minutus* counts occurred under high intensity and indirect control, suggesting that mammalian predator control is beneficial. Differences in the decline of translocated *B. robustus* were observed between moderate and no predator control release sites but could not be unequivocally attributed to predator densities. We recommend replicated predator control studies be undertaken to develop a predator management strategy which will enable grasshopper recovery, and investigate the potential for meso-predator release and prey-switching under regimes that target specific mammals.

Keywords: Orthoptera; Acrididae; *Sigaus minutus*; *Brachaspis robustus*; Mackenzie Basin; New Zealand; predator control; insect conservation

4.5. Introduction

A notable characteristic of New Zealand's historic biota is the absence of certain functional groups, including predatory land mammals (Holdaway 1989, McGlone 2006). Predatory land mammals were first introduced when Polynesians arrived to New Zealand c. AD 1280 and brought with them the kiore (*Rattus exulans*) and the domestic dog (*Canis lupus familiaris*) (Holdaway 1989, Wilmshurst et al. 2008). When European's arrived in the late 1700s, there was a second much larger wave of introductions which included the domestic cat (*Felis catus*), the hedgehog (*Erinaceus europaeus*), and several species of Mustelidae and Rodentia (King 1990). Because the historic fauna of New Zealand did not evolve with predatory land mammals, many native and endemic species lacked defence mechanisms appropriate for avoiding predation by introduced mammals (Daugherty et al. 1993). Consequently, many native and endemic species went extinct after the arrival of predatory land mammals, and many extant species are currently threatened or in decline as a result of their continued presence in native ecosystems (Holdaway 1989, Department of Conservation 2017).

There are several traits seen in New Zealand invertebrates which are associated with vulnerability to predation by introduced mammals, and like other endemic fauna, a number of these traits have been attributed to a lack of co-evolution with mammals (Gibbs 2010). For example, many New Zealand invertebrate species exhibit a freeze response and rely on visual crypsis when threatened (White 1994, Gibbs 1998, Lester et al. 2014). This response provides an effective defence against predators that hunt by sight, such as the native birds and lizards with which many New Zealand invertebrate species co-evolved (Daugherty et al. 1993). However, introduced predatory mammals are often olfactory hunters; therefore, a freeze response when threatened does not prevent detection (Gibbs 1998, Jones et al. 2005, Lester et al. 2014). Other traits that make many New Zealand invertebrate species vulnerable to predation by introduced mammals include flightlessness, living on the ground, and gigantism (Daugherty et al. 1993, Gibbs 1998, Stringer and Hitchmonger 2012). Large invertebrates are particularly vulnerable because they are often preferentially targeted by predatory mammals as a higher value food resource (St Clair 2011, Barker 2016).

Although there has been a recent and major shift in the focus of conservation science in New Zealand to eradicate all introduced mammalian predators by 2050 (*Predator Free 2050*) (Bell 2017, Owens 2017), there are currently no tools available to achieve such large-scale eradication (Department of Conservation 2017, Linklater and Steer 2017). In the interim, significantly reducing the pressure of predatory mammals on threatened or declining species remains a priority of conservation management in New Zealand (Parkes et al. 2017). In some instances, low or moderate intensity predator control is adequate to achieve a conservation benefit (Basse et al. 2003, Whitehead et al.

2008), and is preferred to high intensity predator control because it is more time, cost, and resource efficient. However, mammalian predator control research in New Zealand is routinely focused on the benefit it provides for endemic bird species. For threatened invertebrates, much less is known about the conservation benefit mammalian predator control can provide, let alone the appropriate level of intensity for it to be implemented cost-effectively (Lester et al. 2014).

The distribution of *Sigaus minutus* Bigelow (Orthoptera: Acrididae), an At Risk - Declining grasshopper found throughout the Mackenzie Basin, includes several populations present in areas where mammalian predator control is implemented. Endemic to the South Island of New Zealand, *S. minutus* is a small (males up to 10 mm, females up to 17 mm in body length), rugose grasshopper which is diversely coloured and visually cryptic (Jamieson 1996, Morris 2002, Trewick et al. 2014). Currently, two populations of *S. minutus* exist in locations that receive predator control. The first (Upper Ōhau River) receives direct and high intensity mammalian predator control set up for the protection of black-fronted terns (*Chlidonias albostriatus*) on a braided river island (Woolmore et al. 2010). The second (Tekapō River) receives indirect predator control as a result of prey reduction. It is likely that continuous suppression of rabbits (*Oryctolagus cuniculus*) to very low levels (< 1 rabbit per kilometre) for over more than a decade (Department of Conservation, unpub. data) has resulted in significant reductions to populations of feral cats and ferrets (Pierce 1987, Norbury et al. 2009). As part of annual grasshopper surveys in the Mackenzie Basin, the Department of Conservation has monitored these two populations of *S. minutus* since 2007, along with a third (Lower Ōhau River) in an area of no predator control.

Another Threatened - Nationally Endangered braided river grasshopper, *Brachaspis robustus* Bigelow (Orthoptera: Acrididae), has many of the traits described above that make it susceptible to predation by introduced mammals (Trewick et al. 2014). It is a large (males up to 17 mm and females up to 38 mm in body length), flightless and ground-dwelling insect which relies on visual crypsis when threatened (Bigelow 1967, Morris 2002, Trewick et al. 2014). Body colour varies from shades of pale to dark grey through to reddish-brown allowing it to blend in with its habitat among riverbed gravels and silts. Endemic to the Mackenzie Basin, its range is severely restricted, and the few remaining populations are patchy in distribution and show trends of decline (Department of Conservation, unpub. data). Because diets of predatory mammals in the Mackenzie Basin have been found to contain high proportions of invertebrates (Murphy et al. 2004, Jones et al. 2005, Dowding et al. 2015) it is thought that introduced predatory mammals pose a significant threat to the persistence of *B. robustus*. Currently none of the few remaining natural populations of *B. robustus* receive any mammalian predator control.

Here we evaluate whether there is evidence that reducing mammalian predators is beneficial for threatened and declining grasshoppers in the Mackenzie Basin using two datasets. First, we investigate the long-term population trends of *S. minutus* under the three different predator control regimes; high intensity, indirect through prey reduction, and none. Second, we test whether a moderate intensity mammalian predator control regime is adequate for conservation management for *B. robustus* by translocating wild-caught *B. robustus* to two adjacent locations which differ in their predator management; 1) an area of moderate predator control and 2) an adjacent area of no predator control. The aim of this analysis is to inform conservation managers about appropriate predator management options for protecting threatened and declining grasshoppers.

4.6. Materials and methods

4.6.1. Monitoring of S. minutus

Wild populations of the At Risk - Declining braided river grasshopper *S. minutus* have been monitored by the Department of Conservation at sites in the Upper Ōhau River, Lower Ōhau River and Tekapō River in the Mackenzie Basin (Figure 4.1), as part of annual grasshopper surveys since 2007. All three sites were located on braided riverbed and primarily had a rocky substrate. Ground cover at all sites was comprised of vegetative litter (~4 %), vascular and non-vascular plants, and bare soil and gravel. Non-vascular plants and bare ground made up ~44 % and ~43 % of the ground cover at the Lower Ōhau River site respectively. The Tekapō River site had ~46 % bare ground and vascular plants made up ~32 % of the ground cover. Over half (~57 %) of the ground cover at the Lower Ōhau River site was vascular plants, and ~20 % was bare ground (Department of Conservation 2011, unpub. data). The Upper Ōhau River site receives high intensity predator control targeting seven mammalian species for the protection of black-fronted terns, while the Lower Ōhau River site receives no predator control. The Tekapō River site is situated within the Tekapō Scientific Reserve which receives high intensity rabbit control. Because rabbits are the main prey item for several predatory mammals in the Mackenzie Basin, removal of rabbits can result in reduced presence of predatory mammals (Pierce 1987, Norbury et al. 2002) (Table 4.1). Plot search monitoring at these sites was conducted annually by two to three observers. Observers used a slow walk to systematically search the entire plot on three days within a two-week period each February. The total number of all orthoptera observed during each visit were recorded including *Phaulicridium* spp*., Sigaus '*species A*', S. minutus,* katydids and crickets. The number of each species was recorded along with weather conditions, temperature,

time of day and search duration. No monitoring took place at any site in 2009, the Tekapō River site was not monitored in 2011, and only two visits were made to each site in 2015.

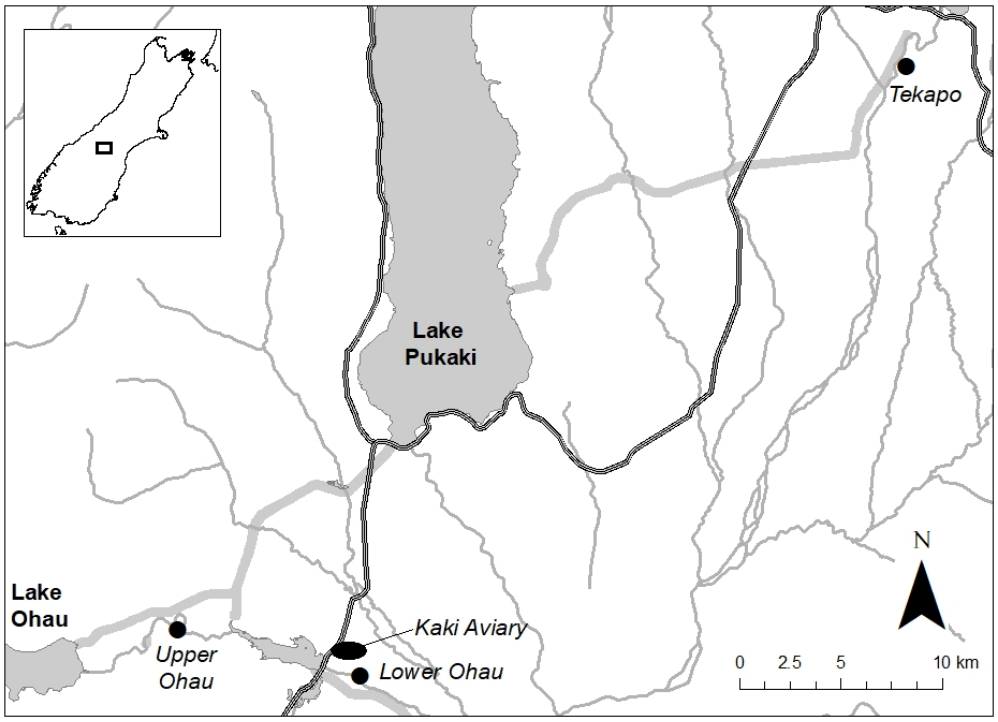

Figure 4.1. The locations of the Upper Ōhau, Lower Ōhau, and Tekapō sites, and the kakī aviary complex in the Mackenzie Basin, New Zealand.

Table 4.1. Descriptions of the predator control implemented at 1) each of the three sites where *S. minutus* is monitored annually by the Department of Conservation and 2) the two areas at the kakī aviary complex which received translocated *B. robustus*.

Species monitored	Location	Predator Control		Monitoring period
		Intensity	Description	
S. minutus	Upper Ōhau	High	In 2010, 448 kill traps (DOC 150 and DOC 250) were deployed over a 1 km diameter (0.79 km^2) grid at the Upper Ōhau River site targeting seven species of mammal (further information in Woolmore et al. (2010)). In 2013, this was supplemented with laced bait to target possum and rat populations (Woolmore et al. 2013).	2007–08, 2010–16
	Lower Ōhau	None	No predator control implemented at the Lower Ōhau River site.	2007–08, 2010–16
	Tekapō	Indirect	The Tekapō Scientific Reserve is a conservation area which is surrounded by a rabbit exclusion fence. There is regular hunting within the reserve for rabbits which have breached the fence. Rabbit presence has been suppressed to < 1 rabbit per kilometre for over a decade (DOC, unpub. data). The presence of predatory mammals whose main prey item is rabbits (cats, stoats, ferrets) is expected to be reduced in this area.	2007–08, 2010, 2012–16
B. robustus	Moderate control area (kakī aviary complex)	Moderate	For almost a decade, 40 kill traps have been deployed within the 0.17 km^2 area which is surrounded by a long-standing high voltage mammalian exclusion fence. In 2015, several un-mended holes in the fence compromised predatory mammal exclusion from the area. An additional 20 kill traps were deployed in the area increasing the total trap number to 60 in September 2015. Occasional hunting for mammals (particularly rabbits and cats) also occurred.	Feb 2015–Feb 2016
	No control area (adjacent to kakī aviary complex)	None	No predator control implemented outside of the predator exclusion fence.	Feb 2015–Feb 2016

4.6.2. Impacts of moderate levels of predator control on translocated B. robustus

In February 2015, a group of 186 *B. robustus* of mixed age, source site, and sex were released into receiving habitats in and adjacent to the kakī/black stilt aviary complex near Twizel (Figure 4.1). Half were released into an area of moderate mammalian predator control and half into an immediately adjacent area absent of predator control (Table 4.1). The moderate control site had a 1.2 m high wire mesh fence that had top and bottom electric fence wires on outriggers, and continuous set kill trapping (DOC250, DOC150 traps) inside the fence boundaries (see Table 4.1). Control was considered 'moderate' because the fence excluded hedgehogs, but only deterred cats and mustelids (this study) and was somewhat 'leaky' for the duration of the study as a result of damage that was not immediately detected.

The presence of mammalian predators was determined for two seasons using tracking tunnels. In March 2015, ten Black Trakka™ tracking tunnels (100 mm x 100 mm x 500 mm) baited with fresh rabbit were run for 25 days in each of the two areas (moderate and no mammalian predator control) to estimate the presence of predatory mammals (excluding cats due to small tunnel size) and lizards. From December 2015 to March 2016, ten larger tracking tunnels (approx. 200 mm x 200 mm x 1100 mm, constructed from black corflute with a wooden base) were run in each of the two areas to estimate the presence of predatory mammals (including cats due to larger tunnel size) and lizards. The tunnels were active for three weeks per month and were baited with peanut butter for the first night (to target mice) and fresh rabbit for the remaining twenty nights (to target larger predatory mammals). Visual assessment of inked footprints was used to identify which predators (cats, hedgehogs, rodents, mustelids, lizards) had passed through a tunnel. The percentage of tunnels tracked by a predator type in each area was calculated for each month the tunnels were active.

Released grasshoppers were monitored fortnightly for a year using mark-recapture until individuals could no longer be detected in February 2016. This coincided with the expected end of the natural lifecycle of the translocated grasshoppers. Release sites consisted of six identical 15 x 15 m gravel plots created on site at the beginning of the study, three inside the predator fence area, and three outside. Gravel plots in the two areas were separated by an average of 150 m. Monitoring was not conducted during the winter of 2015 (May to August) due to cold temperatures and snow fall, conditions under which the grasshoppers were not expected to be active. The number and identity of individuals sighted during each monitoring occasion was recorded and mark-recapture data was used to manually generate estimates of the minimum number of individuals present, taking into account higher counts and sightings of missing individuals on subsequent monitoring occasions.

4.6.3. Data analyses

All analyses were carried out using *R* (R Development Core Team 2011). Annual mean counts of *S. minutus* were modelled using a generalised linear model with a Poisson distribution in the *lme4* package (Bates et al. 2014). In 2007, monitoring was conducted by three first-time observers, rather than two observers including at least one with experience (as per 2008 onwards), and was therefore excluded from analysis. Counts from the Upper Ōhau River were limited to 2010 onwards to coincide with when predator control was implemented. Model fit was assessed using visual assessment for normally distributed residuals. The final model was selected for based on a comparatively low AIC score. Welch's T-tests (to account for unequal variances) were used to compare the percentage of tunnels tracked by each predator type (cats, hedgehogs, rodents, mustelids and lizards) from December 2015 to March 2016 in the moderate and no mammalian predator control areas which received translocated *B. robustus*.

4.7. Results

4.7.1. Trends in S. minutus populations

Under high intensity predator control implemented at the Upper Ōhau River site, *S. minutus* counts showed a significant positive trend over time. On average, counts increased by 10 % per year ($p < 0.001$). A similar trend was seen under the indirect predator control implemented at the Tekapō River site where counts increased significantly by an average of 4.5 % each year ($p < 0.001$). In contrast, at the Lower Ōhau River site where no predator control was implemented, counts significantly decreased by 13 % on average each year ($p < 0.001$) (Figure 4.2).

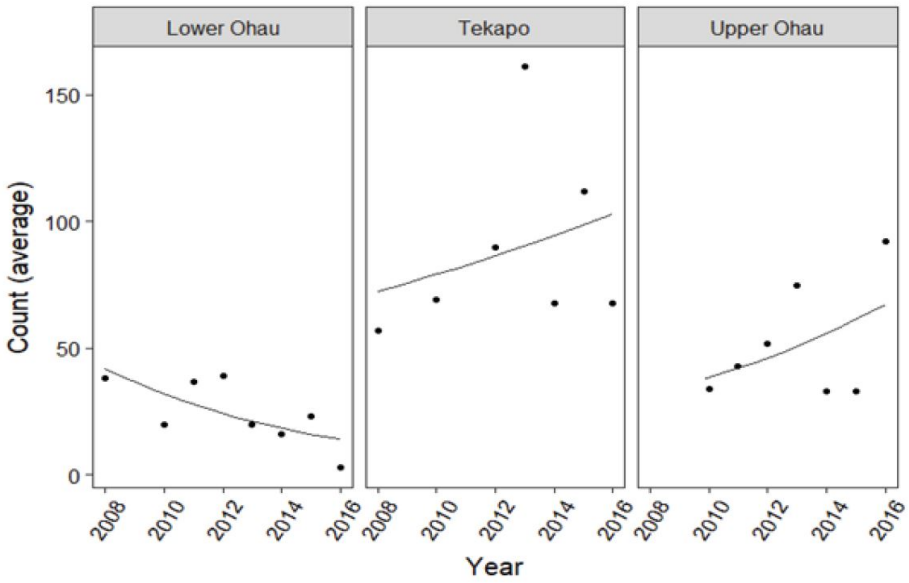

Figure 4.2. Mean count of *S. minutus* over the monitoring period (2008-16) under high (Upper Ōhau) indirect (Tekapō) and no (Lower Ōhau) mammalian predator control regimes.

4.7.2. Experimental translocation of B. robustus

Translocated populations of *B. robustus* in both the moderate control area and no control area showed a steep decline during the first two weeks post-release, with the number of individuals sighted falling to around a third of those released. The number of individuals in the moderate control area then remained stable until the onset of winter, after which there was a gradual decline. In the area with no control, there were two additional distinct periods of decline, once in late autumn and another in late spring (Figure 4.3). Thirteen and eleven individuals were lost over each two-week period respectively.

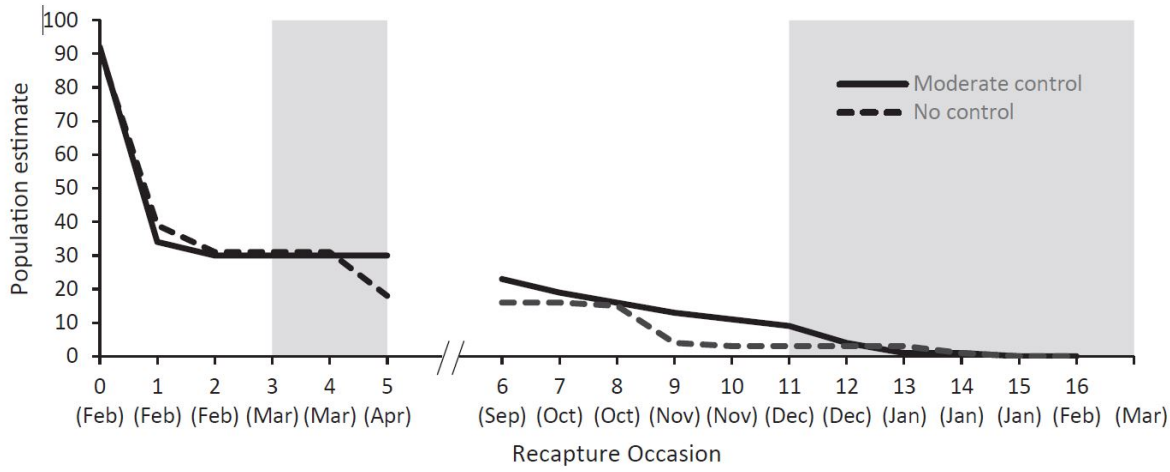

Figure 4.3. The minimum possible number of *B. robustus* individuals present during fortnightly mark-recapture monitoring of translocated grasshoppers in the moderate mammalian predator control area and the adjacent no control area. The break indicates where monitoring stopped for the winter. The grey shading indicates the periods when tracking tunnels were run.

In March 2015, more tunnels were tracked by hedgehogs in the no control area than the moderate control area. In contrast, more tunnels were tracked by rodents, mustelids and lizards in the moderate control area than the no control area (Figure 4.4A). From November 2015 to March 2016, the mean percentage of tunnels tracked by cats, rodents, mustelids and lizards in the moderate and no mammalian predator control areas did not differ significantly (cat, $p = 0.75$, d.f. = 5.3; rodent, $p = 0.67$, d.f. = 4.9; mustelid, $p = 0.63$, d.f.= 4.9; lizard, $p = 0.50$, d.f. = 3.5). The mean percentage of tunnels tracked by hedgehogs was significantly higher in the no control area than the moderate control area ($p = 0.04$, d.f. = 3, Figure 4.4B).

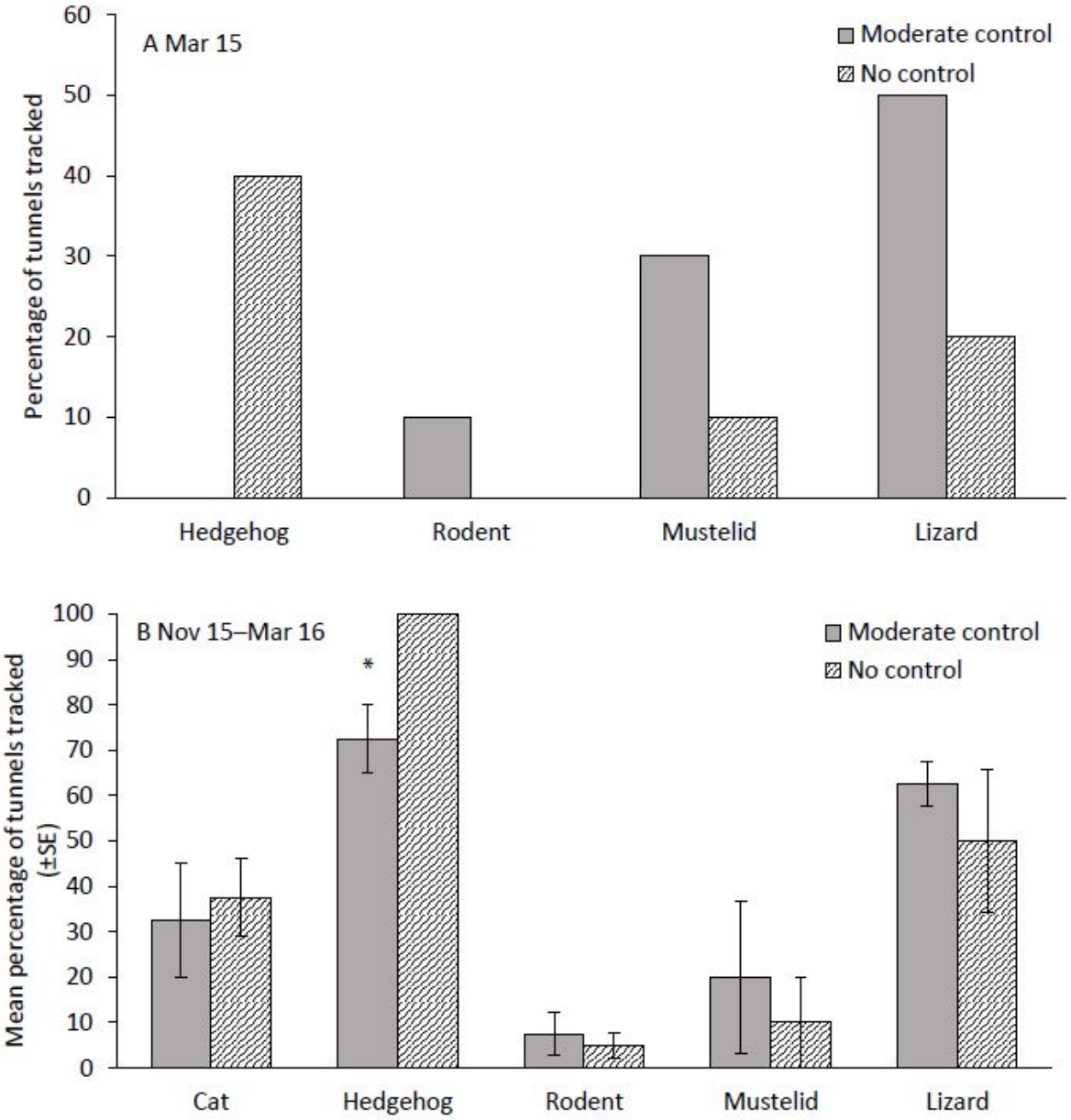

Figure 4.4. Percentage of tunnels tracked by lizards and predatory mammals in the moderate and no mammalian predator control areas that received translocated *B. robustus* grasshoppers in (A) March 2015, and (B) November 2015–March 2016.

4.8. Discussion

4.8.1. Long term trends of S. minutus

The significant increase of *S. minutus* counts under predator control regimes at the Upper Ōhau River and Tekapō River sites suggests that removing predatory mammals from the environment is beneficial

for this At Risk grasshopper, and this is in contrast to the Lower Ōhau River site, where predators were not controlled, and where counts significantly declined over time. However, we did not study the complexity of interactions following the removal of rabbits at the Tekapō Scientific Reserve. The lack of rabbits at this site would not have resulted in a reduced presence of all mammalian predators, only those for which rabbits are the main prey item (i.e., feral cats, stoats and ferrets) (Pierce 1987, Norbury et al. 2002, Norbury et al. 2009). In contrast hedgehog, skink and mice numbers may have been expected to increase following the removal of rabbits due to release from predation by larger predatory mammals, and improved habitat quality and food availability as vegetation recovers. One possible reason for why *S. minutus* numbers may have increased despite predicted increases in hedgehog, skink and mice predators in the reserve, is that the plot where the grasshoppers were monitored is situated on the lower terraces near the Tekapō River. The lower terraces have poorer soils and relatively little vegetation resulting in lower quality habitat relative to other parts of the reserve, which may not support larger populations of hedgehogs, skinks and mice than were present prior to rabbit removal. Future work which explores the potential benefit that mammalian predator control through primary prey reduction could provide should incorporate monitoring of meso-predator populations to better understand responses at various trophic levels.

While the correlation between increasing *S. minutus* populations and low predator densities are compelling, there are several caveats which need to be addressed. The single population monitored under each of the predator control methods (high intensity, indirect, and none) makes it difficult to distinguish the predator control treatment from the location effect. To fully tease apart the predator control effect from the location effect would require long term predator control regimes to be implemented at multiple locations throughout the Mackenzie Basin where *S. minutus* are present. Furthermore, the grasshopper surveys were conducted in February at which time the populations are composed mostly of juveniles. The emergence of *S. minutus* nymphs is likely to be associated with environmental variables such as temperature (Fischer 1994). Variation in the timing of emergence compared to the timing of monitoring may account for some of the year-to-year variability seen in counts of *S. minutus*. Because the life history of *S. minutus* is not well-studied, it is difficult to evaluate exactly how environmental variables may have influenced *S. minutus* counts in this study. A suggested improvement would be to conduct grasshopper counts in December when the population consists mostly of adults. This would result in more accurate and comparable counts year-to-year as adults are larger and therefore easier to count and identify correctly to species. Furthermore, as high mortality rates are often associated with the juvenile stage of insects, counts of adults would be more biologically meaningful.

4.8.2. Experimental translocation of B. robustus

The moderate predator control implemented during the translocation of *B. robustus* was not adequate to achieve an obvious conservation advantage for the species. The steep population decline seen in both the moderate control area and the no control area in the first fortnight following release is assumed to be a normal response to translocation. Many factors that can cause population decline are present during the initial stage of a translocation, such as unfamiliar surroundings leading to dispersal (Heidinger et al. 2009), starvation (if individuals are not able to find adequate food resources in unfamiliar surroundings) and increased vulnerability to predation (when unfamiliar surroundings result in an inability to find appropriate refuges (Jacquot and Solomon 1997, Yoder et al. 2004, Marable et al. 2012)). Stress-induced mortality may also occur as a result of the translocation procedure (Letty et al. 2007, Teixeira et al. 2007, Dickens et al. 2010). Without undertaking the technically difficult task of fine-scale tracking to determine the fate of individual grasshoppers, it was not possible to tease out the relative importance of these factors in the initial decline observed in this particular translocation.

After the initial decline, grasshoppers in the no control area had two episodes where the minimum estimated number of individuals present dropped by 13 and 11 individuals in a fortnight (between March - April and October - November respectively). No such steep declines were seen in the moderate control area where the maximum number of individuals lost over any single fortnight was estimated at no more than five. Given that the initial population decline appears to have stabilised before these steep decline events in the no control area, one explanation is that they were a result of hedgehog predation. A higher tracking rate of hedgehogs was detected in the no control area and hedgehogs are known to exploit food patches, meaning they will stay in a resource-rich area and remove large numbers of prey in a short amount of time (Jones et al. 2005). We note, however, that if predation was a key factor explaining declines, it did not result in all grasshoppers being completely extirpated from non-predator managed sites.

Several challenges remain in understanding the population trends observed following the *B. robustus* translocation. In particular, two key challenges are to better determine the fate of released individuals, and to better understand the roles of different species of mammalian predators. Post-release fate tracking is both a technically challenging and time-consuming endeavour for grasshoppers because they moult several times over the course of a spring-autumn period. The mark-recapture tracking used during these translocations was only effective at determining the fate of individuals that remained alive and stayed within or immediately adjacent to the plots. The small body size and highly cryptic nature of *B. robustus* makes them difficult to detect visually, and dead bodies, even if present,

were never recovered to determine cause of death. Tracking using telemetric devices attached to the pronotum could have been used to partially overcome this problem (Kissling et al. 2014). However, most individuals were mid- to late-instar nymphs rather than adults at the time of translocation and subsequent moults would have meant any telemetric devices would have been shed along with the exoskeleton, if the device itself did not prevent successful moulting in the first instance.

4.8.3. The differential roles of key mammalian predator species

It is unlikely that all mammals pose an equal threat to grasshoppers. Because the predator control programme in the high intensity site (Upper Ōhau River) used a range of tools to control seven species of mammalian predators (excluding mice) (Woolmore et al. 2013), it remains unclear whether any one or a subset of these predators are key in suppressing *S. minutus* populations. However, the absence of hedgehogs within the moderate control area suggests they are one of the key predators of *B. robustus*.

Hedgehogs are present in higher abundance than any other predatory mammal throughout the Mackenzie Basin (Keedwell and Brown 2001), and exhibit a diet comprised mostly of invertebrates (Moss and Sanders 2001). Scat analysis has shown grasshoppers (White 1994), and other orthoptera (Jones et al. 2005) form a substantial component of the hedgehog diet in the Mackenzie Basin. Of concern is their ability to consume numerous invertebrates in a short space of time. Jones et al. (2005) found hedgehogs could exploit rich food patches by consuming large numbers of individuals in a single night. For Mackenzie Basin grasshoppers, which tend to have a patchy distribution and loose congregations, this behaviour is particularly threatening.

While invertebrates are not the primary prey of cats, they do make up a substantial proportion of their diet and are a common alternative prey item. In the Mackenzie Basin, invertebrates have been found in 10 - 30 % of cat guts and scats (Pierce 1987, Murphy et al. 2004). Grasshopper remains have also been found in cat scats both in the Mackenzie Basin (Pierce 1987, White 1994) and other parts of New Zealand (O'Donnell et al. 2017). In years when cats' main prey, rabbits, have experienced population crashes due to poisoning, the percentage of cat guts containing invertebrates increased to around 50 %, suggesting a switch to smaller available prey (Murphy et al. 2004). White (1994) suggested this prey switching caused the loss of marked individuals in his study of *B. robustus* in 1991–92. Even when rabbit prey are available, the percentage of cat guts containing invertebrates is still higher in the Mackenzie Basin than for other ecosystems in New Zealand; in other grassland regions between 5 % and 10 % of cat guts contain invertebrates (Langham 1990) while in forests it is < 1 %

(Harper 2005). It is likely that kittens are a bigger threat to grasshoppers than adult cats as they are often too small to catch rabbits so must rely on smaller prey (Gillies 2001).

Although stoats have been found to consume significantly fewer invertebrates than cats, their diets still contain a substantial proportion of invertebrates (Murphy et al. 2004). Like cats, there is evidence that stoats will prey-switch and consume more invertebrates when rabbit densities are low (Murphy et al. 2004). The same occurs with ferrets, however ferrets have been found to consume fewer invertebrates than both stoats and cats (Murphy et al. 2004). For both stoats and ferrets, it is the smaller females which consume the most invertebrates and therefore pose the biggest threat to the grasshoppers (Pierce 1987, Dowding et al. 2015). The low abundance of stoats in this ecosystem (Keedwell and Brown 2001) and the low consumption of invertebrates expected from ferrets suggests the threat of mustelids to Mackenzie Basin grasshoppers is likely to be small.

Little is known about the diet of rodents in the Mackenzie Basin, however studies on offshore islands have revealed that most often their presence has negative impacts (decline or suppression) on invertebrate populations (St Clair 2011). On the mainland, kiore and ship rats (*Rattus rattus*) have negatively impacted populations of New Zealand wētā species (*Hemideina*) and appear to threaten the largest individuals most strongly (Rufaut and Gibbs 2003, Ruscoe et al. 2013). All rats identified in the Mackenzie Basin area have been Norway rats (Department of Conservation, unpub. data). Keedwell and Brown (2001) found the catch trapping rate for rats in the Ōhau, Tekapō and Pūkaki Rivers to be the second lowest (to stoats) of the key predatory mammals. The low abundance of rats in this ecosystem means their threat to grasshopper populations is likely to be small. Less is known about the abundance of mice in the Mackenzie Basin, but there are no reported observations of large scale eruptions in the last three decades, and only three mice were detected in over 2700 days of video monitoring at bird nests in several Mackenzie Basin braided rivers (Sanders and Maloney 2002). In snow tussock ecosystems in Fiordland, grasshoppers can make up 13 % of the alpine mouse diet (Wilson and Lee 2010). If mice were abundant in braided river ecosystems, they could be threatening to the persistence of Mackenzie Basin grasshoppers.

In braided river ecosystems in the Mackenzie Basin, introduced predatory mammals are top terrestrial predators (Murphy et al. 2004). Control activities, or eradication initiatives such as *Predator Free 2050*, which aim to remove some, or all, introduced mammalian predators from the ecosystem could result in a meso-predator release (Soulé et al. 1988, Linklater and Steer 2017). The consequence being an increase in the abundance of grasshopper predators at lower tropic levels; particularly birds and lizards, and possibly predatory invertebrates such as spiders (Norbury et al. 2013, Watts et al. 2014). At the translocation sites for *B. robustus*, the tracking rate of skinks in the moderate control area was found to be greater than in the no control area, suggesting that a meso-predator release

may have occurred at that site. Because many of the bird and lizard species in this ecosystem are also threatened or in decline (Department of Conservation 2017), it is not expected that removal of predatory mammals will result in stronger predation pressure from non-mammalian predators than was historically present. However, it is not well understood how populations of mammalian predators at lower trophic levels, such as mice or stoats, might respond to the removal of apex predatory mammals in these ecosystems (Pierce 1987, Keedwell and Brown 2001). A better understanding of these types of interactions is needed to ensure control or eradication of a sub-set of mammals does not have unintended negative consequences for populations of threatened invertebrates.

4.9. Conclusions

We provide evidence here that the high intensity predator control undertaken for black-fronted tern protection was correlated with a significant increase in *S. minutus* counts over time, as did indirect predator control through prey reduction. This suggests that predatory mammals do limit *S. minutus* population growth. Differences in the decline of translocated *B. robustus* individuals were observed between moderate and no predator control release sites but could not be unequivocally attributed to predator densities due to challenges in determining the fate of released individuals. Although further replicates would likely generate stronger support for our findings, we suggest that a more pressing priority is to determine which control strategy is most beneficial. Even though *S. minutus* appeared to benefit from indirect predator control, we caution that it may not be an effective conservation strategy for all threatened or declining grasshopper species because prey-switching is known to occur in some of the key predatory mammals (Keedwell and Brown 2001, Norbury 2001). In particular, large insect species like *B. robustus* may be more strongly targeted by predatory mammals as an alternative prey item because they provide a higher energetic benefit than small insects (Pyke et al. 1977). If prey reduction is implemented as a management technique, we recommend that predatory mammals be controlled simultaneously to reduce the chance of prey-switching to invertebrates (Keedwell and Brown 2001).

Because predator control implemented both in the Upper Ōhau River and the moderate control area of the translocation were designed to control for introduced mammals in general, it remains unclear whether any one or several of these predators are key in suppressing grasshopper populations. It is also unclear whether *B. robustus* is more vulnerable to predatory mammals than the much smaller *S. minutus*. To determine exactly how to protect threatened and declining grasshoppers from introduced mammalian predators, further study is needed to identify the intensity of control required, the predators for which management is needed, and any unintended increases in predation

pressure caused by meso-predator release or prey-switching (Soulé et al. 1988, Norbury et al. 2013, Watts et al. 2014). Given the threat status of these two Mackenzie Basin grasshoppers, however, we suggest that their future conservation management should prioritise high intensity mammalian predator control for the full suite of predators.

4.10. References

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Chapter 5 Informing the design of a long-term monitoring protocol for a highly cryptic Nationally Endangered insect: Removal sampling as a basis for protocol development

5.1. Preface

Understanding life history, requirements for growth and development, and how to select an appropriate receiving habitat are important considerations to make prior to conducting a translocation of a threatened species. However, after a translocation has taken place it is equally important that outcomes for the population are accurately measured. This is essential because it allows any causes of failure to be identified, and adjustments to translocation protocol to be made to improve future success (Parker et al. 2013). In the Introduction of this thesis, several aspects of the current monitoring method for *B. robustus* were identified as potentially leading to false representation of population trend, the most notable being the poor visual detection of grasshoppers by an observer (White 1994, Fraser 1999). This chapter is the first of two chapters that focus on developing an effective monitoring strategy for *B. robustus*. Here, intensive removal sampling during a single active season (November to March) is used to rapidly quantify seasonal and demographic variation in visual detectability of *B. robustus*. The results presented here provide guidelines for maximising visual detection of *B. robustus* grasshoppers during population monitoring.

A version of this chapter has been submitted to the *Journal of Insect Conservation*.

Informing the design of a long-term monitoring protocol for a highly cryptic Nationally Endangered insect: Removal sampling as a basis for protocol development

Schori, J.C.[1], Steeves, T.E.[1] and Murray, T.J.[2]

1. School of Biological Sciences, University of Canterbury
2. School of Forestry, University of Canterbury

5.2. Abstract

Imperfect detection of individuals in threatened wild populations is common and can obscure real population trends when it is unaccounted for in population monitoring, and therefore impede conservation decision making. For many threatened insects, there is a lack of biological information or available long-term data to inform how best to practice data collection and population monitoring. Here, we inform the design of a future long-term population monitoring protocol for *Brachaspis robustus*, a Nationally Endangered grasshopper endemic to the Mackenzie Basin in New Zealand. We use removal sampling during a single active season (November to March) to rapidly quantify seasonal and demographic visual detectability. Juvenile instars dominated population composition in all months except December and males represented > 50 % of monthly captures. Adult females were 2-3 x larger than adult males, and 79 % of those captured were found during the first search of an area compared to only 52 % of adult males. The odds of detecting an individual increased by 6 % per 1 mm of body length. Removal sampling was found to be an effective method for rapidly informing future long-term monitoring design for a cryptic, threatened insect. Recommendations for long-term population monitoring throughout the species' range include monitoring adult females as an index of population size, restricting monitoring to when adult abundance is at its peak (November and December), and conducting multiple monitoring events within peak months to counter the effects of daily and seasonal variation and imperfect detection.

Key words: removal sampling; conservation; monitoring; *Brachaspis robustus*; detectability.

5.3. Introduction

Population monitoring is crucial for measuring the decline and/or recovery of threatened species (Campbell et al 2002). Its applications include evaluating the outcomes of conservation management action (Lyons et al. 2008), detecting sudden or rapid population decline in response to stochastic events or lack of appropriate management, and invoking action when population size or trend declines below desirable levels (Block et al. 2001, Cook et al. 2016). However, the accuracy of population size estimates, and therefore the identification of real trends, can be obscured when the detection of the species is imperfect or variable in space or time. When imperfect or variable detection is unaccounted for in monitoring design it can lead to poor data interpretation and potentially impede informed conservation decision making.

There are several reasons why monitoring of threatened insects might produce inaccurate estimates of population size or trend. Insects are almost never perfectly detected during monitoring events, and detection probabilities can change with the weather, individual size or colour, and the time of day that monitoring is conducted (Dennis et al. 2006, Harker and Shreeve 2008, Driscoll 2010, Hudgins et al. 2012). In addition, insects typically produce large numbers of offspring with high rates of juvenile mortality (Dempster 1963), often resulting in rapid fluctuations in population size that do not necessarily reflect a long-term population trend. Conducting monitoring during periods of high detection can improve data quality (Driscoll 2010), or quantifying detection probability itself can be used to correct counts and improve data interpretation (White et al. 2015). However, for many threatened insects there is a lack of basic biological information and long-term population data are rarely available (Braby 2018) for predicting periods of high detectability or informing how to maximise data quality.

For some species, removal sampling can be useful for quantifying detectability, and when repeated over time, also used to evaluate seasonal detectability trends. Removal sampling is an intensive monitoring technique that captures absolute density estimates of closed populations (Clark et al. 1995). It is often not suitable for long-term monitoring because it is invasive and labour intensive, and other methods that record a representative subset of the population (e.g. transect counts, mark-recapture) are usually preferred (Pollock et al. 2002). However, a short period of sampling does have the potential to rapidly generate a dataset that can be used to inform the *design* of long-term population monitoring protocols for threatened insects when long-term data are absent.

The grasshopper *Brachaspis robustus* Bigelow (Orthoptera: Acrididae) is a Nationally Endangered grasshopper (Trewick et al. 2014) endemic to the Mackenzie Basin (~7,339.23 km^2) in the centre of New Zealand's South Island (White 1994). This species is restricted to braided riverbeds and

associated terraces throughout the basin, and is found below ~800 m a.s.l. (Bigelow 1967, White 1994). Adult females are large (body length up to 36 mm) compared to adult males (up to 17 mm long). As a non-stridulating species, *B. robustus* is acoustically cryptic. Both sexes are also cryptically coloured in pale to dark grey or green-brown that mimics the rocky and silty substrates of their habitat. Visual detection for monitoring generally requires the grasshopper to jump in response to the approach of an observer (White 1994, Fraser 1999), however, no studies have quantified the proportion of the population that is detected using this method. Furthermore, very little is known about the population demographics of *B. robustus* or how they change seasonally, except that the species is expected to have a life span > 2 years (Chapter 2), and to be able to overwinter at most life stages (White 1994). The development of an efficient quantitative monitoring protocol that effectively estimates a biologically meaningful population size and trend over time is required to enhance conservation management of *B. robustus*.

Thus, the aim of this study is to quantify the detectability of *B. robustus* using data collected during removal sampling over a single active season (November to March) for the purpose of informing the design of a future monitoring protocol. The key objectives of this study are to 1) record changes to the demographic composition of the population through the active season; 2) assess the distribution and density of individuals in space relative to environmental variables; 3) quantify detectability and determine how it varies with sampling time, grasshopper sex, and grasshopper body size; 4) make recommendations that inform the design of a future long-term monitoring protocol for *B. robustus*; and 5) provide a basis for developing monitoring protocols for similarly cryptic invertebrates.

5.4. Methods

5.4.1. Site descriptions

This study was conducted at Patersons Terrace, a site located ~8 km SW of the town of Tekapō, where one of the largest and most stable populations of *B. robustus* inhabits an un-used gravel road running parallel to the Tekapō Canal (Figure 5.1). The road, originally established during the construction of the Tekapō Canal in the 1970s (McKay et al. 1978), is on average 5.4 m wide and consists of gravels and cobbles thought to have been sourced from the Tekapō River. The road has been fenced off from regular vehicle use for several decades, however until recently domesticated sheep (*Ovis aries*) and introduced rabbits (*Oryctolagus cuniculus*) and hares (*Lepus europaeus*) grazed the semi-modified grasslands that border the road (Department of Conservation 2004). The region has a relatively

continental climate with average daily temperatures of 15 °C in summer and 3 °C in winter, although they often rise above 30 °C and regularly fall below 0 °C (Macara 2016). The area is characteristically dry receiving an average rainfall of < 600 mL per annum (Macara 2016).

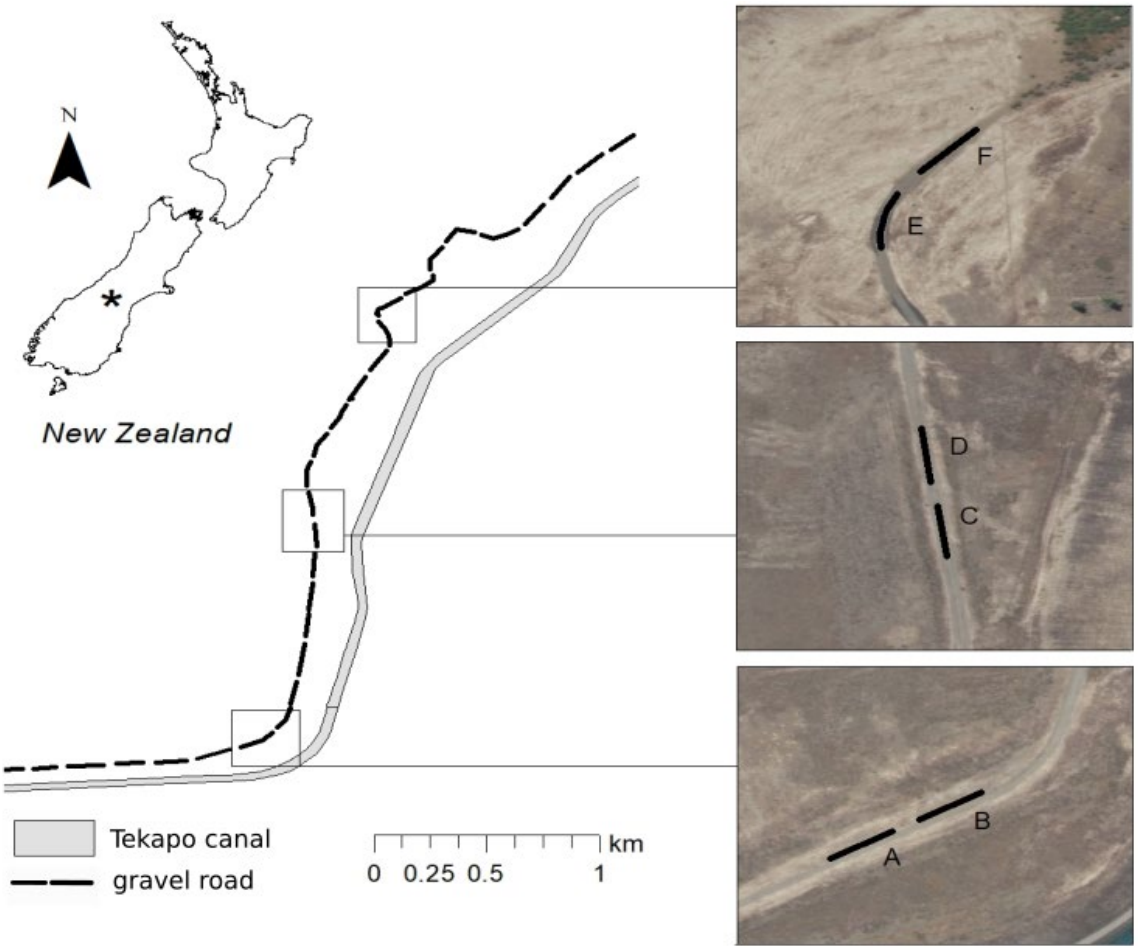

Figure 5.1. The location of the 6 plots (A-F) situated on an un-used gravel road that runs parallel to the Tekapō canal through semi-modified grasslands. The site is located ~8 km SW of Tekapō township within the Mackenzie Basin (indicated by asterisk on map) in the central South Island, New Zealand. (Aerial imagery sourced from LINZ Data Service and licensed for re-use under the Creative Commons Attribution 3.0 New Zealand licence.)

5.4.2. Field methods

Six plots (A, B, C, D, E and F from south to north) were set up along the un-used gravel road (Figure 5.1). Plots were 40 m long, occupied the width of the gravel road (~5.4 m, estimated plot area of 216 m^2) and were spaced > 20 m apart. Grasshoppers do not generally leave the gravel area making the grassland on either side of the road effectively a closed edge (Chapter 3) such that only the two short ends of the plot (11.9 % of the perimeter) were open to grasshopper movement into and out of the

plot. Ground cover composition of each plot was estimated using a 1 m^2 quadrat, divided into 100 equal squares. The quadrat was placed at 10 m intervals along the centre of the road and a visual estimate of ground cover composition for each quadrat was recorded using the following categories; gravel, mat daisies (*Raoulia australis*), hawkweed (*Hieracium* spp. and *Pilosella* spp.), rabbit faeces, and 'other' (all small, un-identified herbaceous plants).

Before removal sampling of *B. robustus* began, barometric pressure, air temperature (1 m above ground), and ground surface temperature (in the shade and sun) were measured using a Kestrel 3500 Pocket Weather Metre (GeoSystems New Zealand LTD) which was only available from December onwards. Cloud cover (categories: none; high cloud, when cloud was high in the sky but did not cause shadows; patchy cloud, when clouds were lower in the sky and caused shadows; overcast) and wind conditions (categories: none, low winds, high winds) were recorded at the start and end time of each search. Removal sampling consisted of a single observer first searching a 1 m wide transect lengthwise down the centre of the plot. Then the observer walked forty 1 m wide transects back and forth across the width of the plot (road edge to road edge). The direction of the search (i.e. north to south, or south to north) was chosen to ensure the shadow of the observer fell as much as possible in the area that had already been searched. This was found to improve sightings of grasshoppers because the observer's eyes did not have to adjust between the contrast of the highly reflective substrate and their own dark shadow when the grasshopper moved. The walk was slow, and the observer's front leg was held low to the ground and gently waved back and forth to illicit a jump response making visual detection of grasshoppers possible (Figure 5.2). Any *B. robustus* grasshopper detected was captured by hand, placed in a labelled holding-tube and stored in a chilly bin (an insulated bin half filled with ice packs) in a shaded spot on the side of the road. A flag was placed on the road to mark the location where each grasshopper was first sighted. Flags were constructed of a small metal tent peg and flagging tape labelled with the search number (e.g. "Search 1") and the individual's number (e.g. '#12'), corresponding to the label on the holding tube. The locations of the flags were recorded using a GPS (Garmin E-trex 20). Once the entire area had been searched, the width wise search process (as described above) was repeated no sooner than half an hour after the previous search had begun. This was continued until either no more individuals were found, or five searches of the plot had been completed. Captured individuals were measured for body length (from the tip of the head to the end of the abdomen) and hind femur length, and sex and colour were recorded. Because accurate methods to determine when an individual had reached adulthood had not been developed at the time of this study, "adulthood" was based on measured femur length. Females were considered "adult" if their femur was ≥ 15 mm, and males were considered "adult" if their femur was ≥ 9 mm. Juveniles with a

body length < 8 mm were not included in this study because of a high risk of fatal injury to the grasshopper during capture.

Figure 5.2. The author (J.C.S) searching for *B. robustus* at Patersons Terrace. The front leg is held low to the ground and waved gently back and forth, and the author's shadow falls into the area of road that has already been searched.

The removal study was conducted three times a month in each plot for five months from November 2015 until March 2016 (total of ninety plot searches). Due to time constraints, the final day of sampling for 'January' occurred on the first day of February. In December, plot A was only sampled twice, and plot C was sampled four times. Sampling was only conducted on days when weather conditions were favourable for grasshopper detection and observer safety (i.e. not conducted when air temperatures were < 14 °C nor during fog, rain, high winds or electrical storms). Searches began no earlier than 9 am to ensure sufficient time for the ground temperature to warm above the threshold for grasshopper activity. On warm days, searching could end as late as 6 pm.

5.4.3. Data analyses

All statistical analyses were carried out in *R* (R Development Core Team 2011), unless otherwise stated. ANOSIM in *vegan* (Oksanen et al. 2018) was used to test for differences in ground cover composition between the plots, followed by pairwise comparisons using *pairwise.adonis* (Martinez Arbizu 2019). Mean plot temperatures over the sampling period were compared using ANOVA. ANOVA and Tukey's

HSD were used to conduct pairwise comparisons of the mean density of grasshoppers in the six plots within months, and compare density changes within individual plots over the five months, and to compare the body lengths and femur lengths of juvenile males and females across the five sampling months. Because data was discrete and did not meet the assumption of normality, Kendall's rank correlation 'τ' was used to test for significant correlation between femur length and body length for juvenile male and female grasshoppers. The probability of detecting an individual (p_g) in the first search (compared to not being detected when known to be present in the plot, i.e. detected in a subsequent search) was modelled with a binomial linear mixed-effect model in *lme4* (Bates et al. 2015) using a logit link function and *BOBYQA* optimisation (Powell 2009). Any grasshoppers found in the first lengthwise transect search of the plot were excluded from the dataset to ensure search area was comparable between searches. Given the short duration of each plot search, and the relatively small portion of the plot perimeter open to grasshopper movement, it was assumed that the population within each plot was demographically closed during the search period. Cloud cover (levels: no cloud, high cloud, patchy cloud, overcast), month, sex and body length (mm) were explored as fixed effects, and Plot (levels: A, B, C, D, E, F) was specified as a random effect. Because femur length was found to be correlated to body length, and because body length was the attribute seen by an observer during a grasshopper detection event, femur length was not included in the model. Model fit was assessed by checking for the absence of overdispersion, and visual assessment for normal distribution of residuals and homogeneity of variance. Model selection was conducted using ANOVA to determine significance of fixed effects in nested models and selecting for a low AIC (Akaike Information Criteria). Time spent searching was not included as a variable because search time increased as the number of grasshoppers to catch increased. Instead it was assumed that equal search effort was executed at each search because an equal amount of surface area was searched by the observer using the same method on each occasion.

5.5. Results

5.5.1. Plot descriptions

The ground cover within the plots consisted of stones (gravel and cobbles), mat daisies (*R. australis*), grasses, rabbit faeces, hawkweed, mosses and small herbs. Lichens occurred on most rock surfaces but were not measured in this study. Plot A had the lowest gravel cover (52 %) and the highest coverage of mat daisies (31 %) and rabbit droppings (6 %). All other plots had ≥ 88 % gravel cover (Figure 5.3). Mat daisies were absent from plots C, D, and E, and rabbit droppings were not found in

plots B, E and F. A statistically significant difference in ground cover between plots was detected but differences were marginal (ANOSIM, R = 0.21, p = 0.001) and pairwise comparisons did not reveal any significant differences. Mean temperature during plot searches throughout the active season did not differ significantly between plots (F (5,60) = 0.034, p = 0.99, Figure 5.3). Plots A, B, E and F had a NE-SW orientation, and plots C and D had N-S orientation. Plots A and B had a southward facing aspect, but plots C, D, E and F were level. Plots C and D were the most sheltered, having gently raised banks of approximately 1 m on either side of the plot. Although soil moisture was not assessed, plot E was observed as the dampest of the plots. After heavy rainfall, ~5 m^2 (or ~2 %) of the plot was inundated by a puddle that remained for several days depending on subsequent weather conditions. All other plots were free draining.

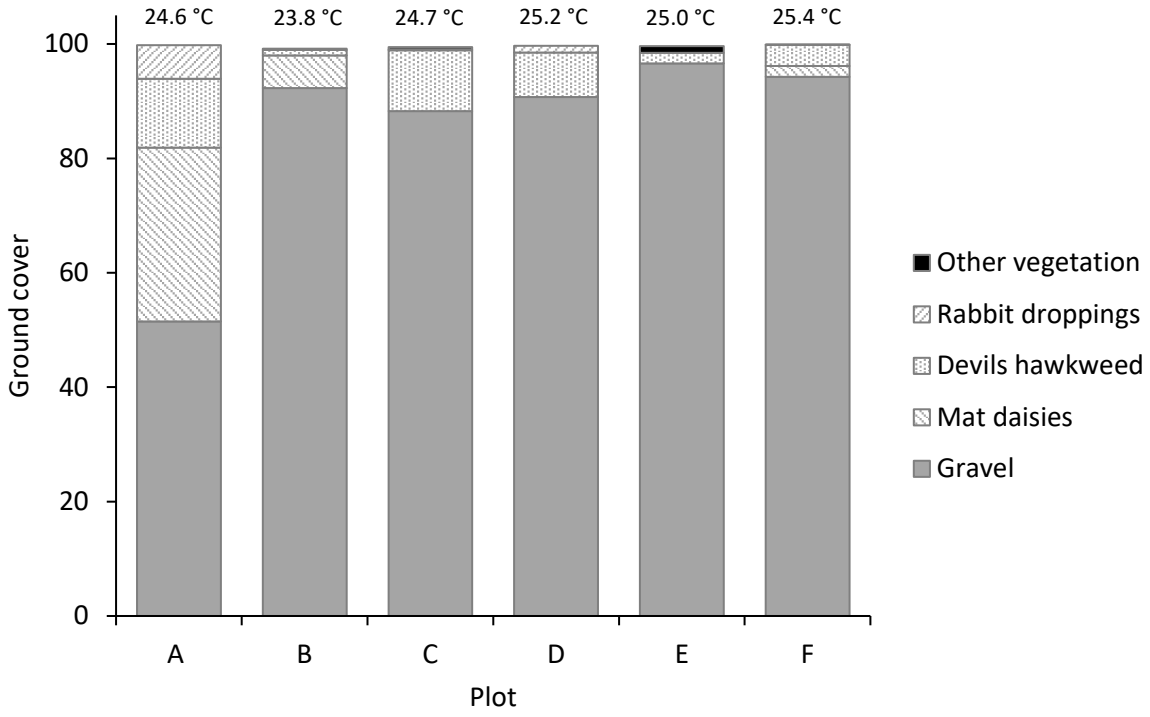

Figure 5.3. The estimated ground cover composition (%) in each of the six plots used in the removal study. The mean ground temperature (°C, in the shade) during search periods is indicated above each bar.

5.5.2. Population demographics

A total of 1,486 grasshopper captures were made during 90 search and remove events conducted over 53 days between 1 November 2015 and 20 March 2016, this included 51 captures of adult females and 214 of adult males. Sixty-nine percent of captures in December were of adults, while the majority of captures in all other months were of juveniles (Figure 5.4). In all months, more males were captured

than females (November, 59 %; December, 57 %; January, 53 %; February, 66 %; March, 68%, Figure 5.4). Juvenile body length was longest in December and shortest in January (Figure 5.5) and strongly correlated with body length for both sexes (males, $\tau = 0.68$, $p < 0.01$; females, $\tau = 0.75$, $p < 0.001$). Femur length showed the same trend (Figure 5.5). For adults, the mean body length of females was 34 (± 0.5) mm and of males was 17 (± 0.1) mm.

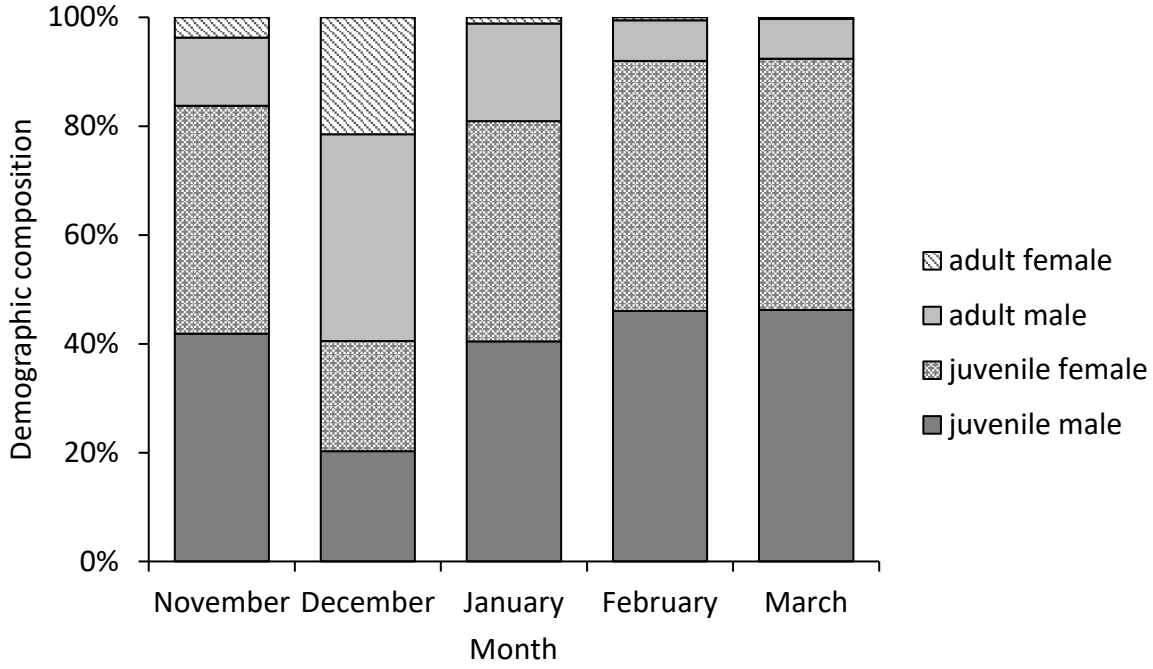

Figure 5.4. The total demographic composition of *B. robustus* detected in six plots included in the search and remove study from November 2015 to March 2016. Number of grasshoppers (*n*); November *n* = 176, December *n* = 141, January *n* = 420, February, *n* = 440, March, *n* = 309.

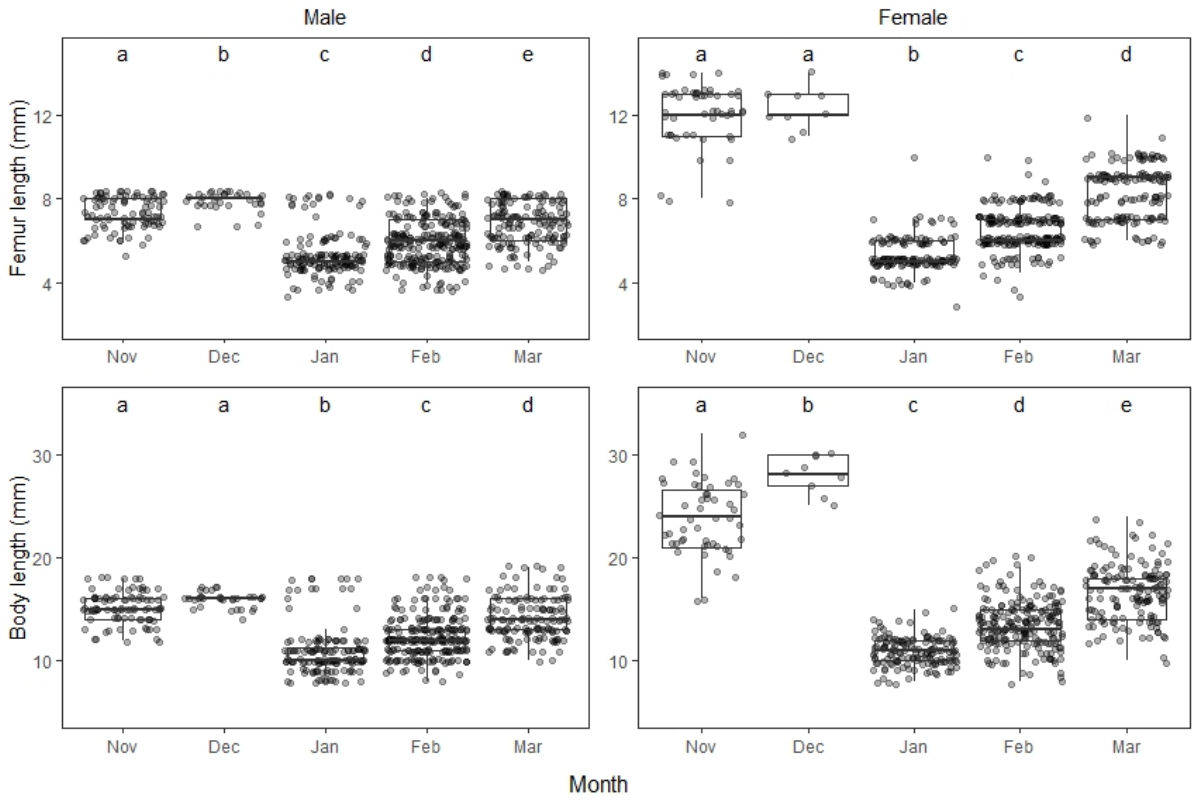

Figure 5.5. The distribution of *B. robustus* juvenile male (left) and female (right) femur length (mm), and body length (mm, measured from the top of the head to the tip of the abdomen) between November 2015 and March 2016. Common letters denote non-significant difference within groups (Tukey's HSD, $p > 0.05$).

5.5.3. Population density

The number of individuals (adults and juveniles combined) found in a single plot during a single search and remove event ranged from 1 (December, plot E, density = 0.5 individuals per 100 m^2) to 54 (February, plot F, density = 25 individuals per 100 m^2). Within months, plot F had the highest mean grasshopper density throughout the entire active season and peaked in January (Figure 5.6).

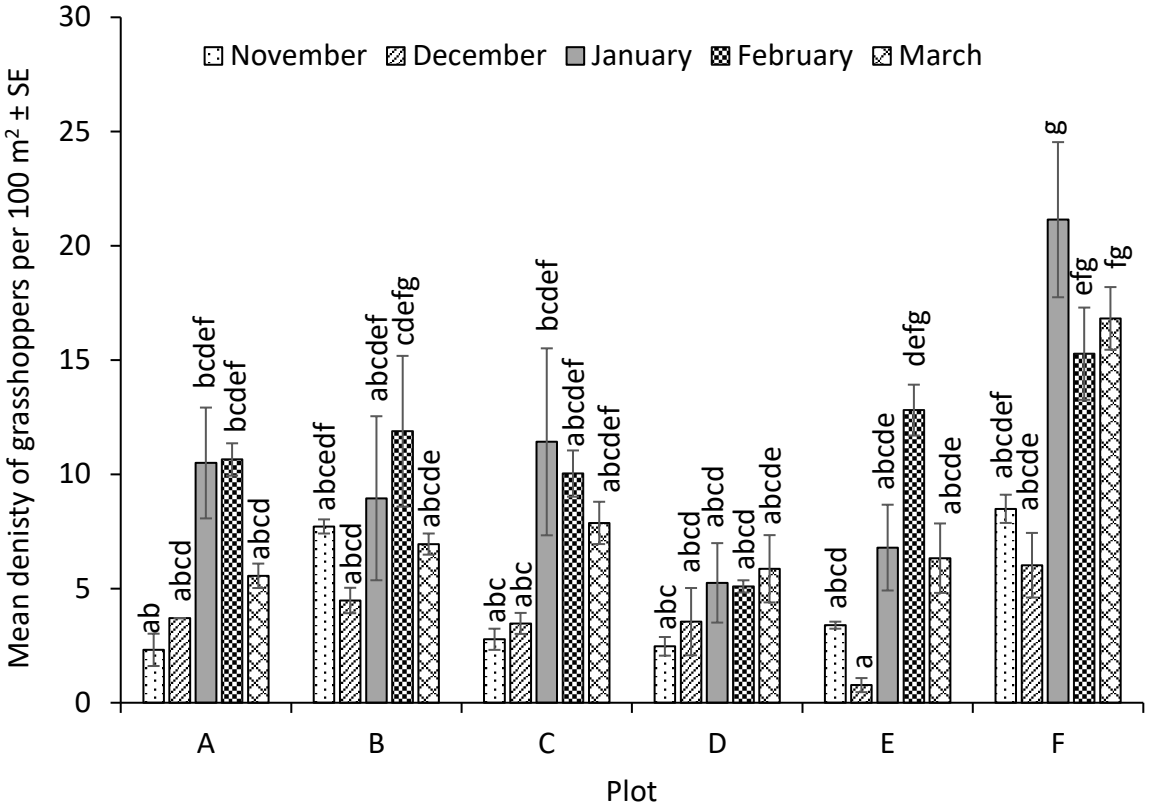

Figure 5.6. The mean density (± SE) of *B. robustus* (including all adults and juveniles) per 100 m^2 in each plot for each month (*n* = 3 sampling days per month). Bars with common letters do not significantly differ (Tukey's HSD, $p > 0.05$).

The highest number of adult females and adult males found in a single plot was four and ten respectively, both in December (density; females = 1.85, and males = 4.63 individuals per 100 m^2). Adult mean density peaked in December for females and January for males. For juveniles, both sexes peaked in February (Figure 5.7).

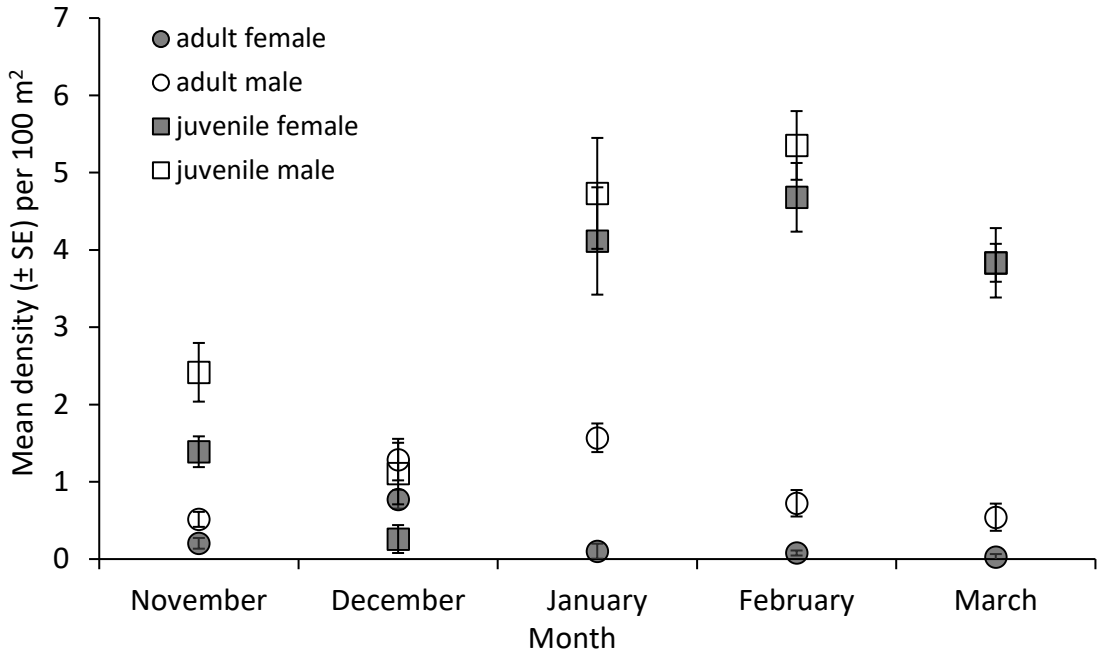

Figure 5.7. The mean density (± SE) of adult and juvenile male, and adult and juvenile female *B. robustus* per 100 m^2 across the monitoring period (November to March), based on 18 count events per month.

5.5.4. Detectability

Seventy-nine percent of adult female grasshoppers that were captured in this study were found during the first search of a plot compared to only 52 % of adult males. For the juvenile instars, the proportion of individuals captured during the first search of a plot varied by month. For juvenile females, the highest proportion captured in the first search was in December (88 %) followed by November (70 %), February (50 %), March (50 %), and January (49 %). For juvenile males, the highest proportion captured on the first search was in November (69 %) followed by December (60 %), February (46 %), March (46 %), and January (38 %). There was no evidence that the odds of detecting an individual that was present in the plot was affected by sex or cloud cover, and these variables were not included in the final model. Odds increased by 6 % (± 2 % SE) with every additional 1 mm of body length ($p < 0.001$). When length was fixed, the odds of detecting an individual in December ($p_g = 0.74$) did not significantly differ from November ($p_g = 0.81$, $p = 0.44$), but were significantly higher than January ($p_g = 0.55$, $p = 0.02$), February ($p_g = 0.51$, $p = 0.007$) and March ($p_g = 0.50$, $p = 0.005$, Figure 5.8).

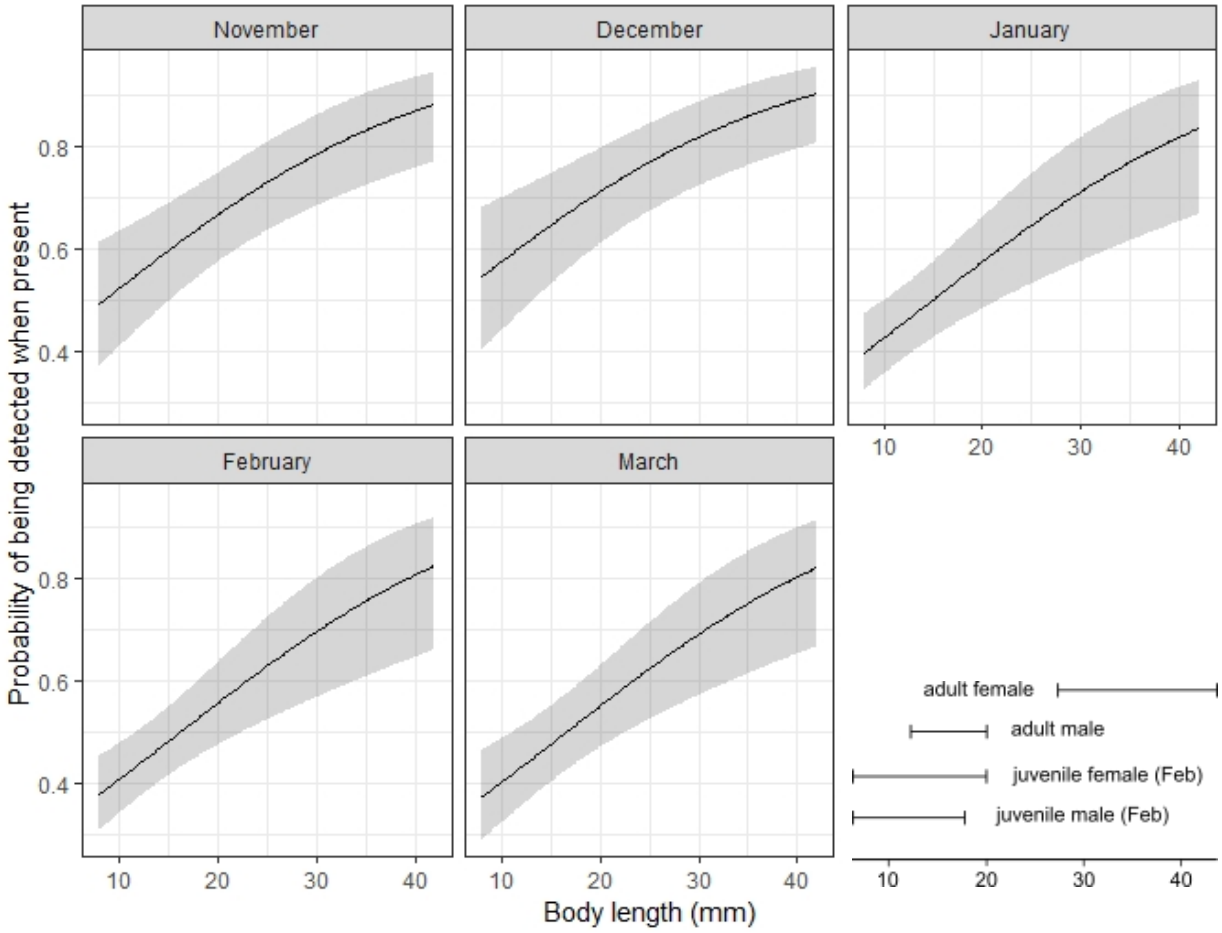

Figure 5.8. The probability (± SE) of a *B. robustus* individual being detected when it is present in a plot relative to the individual's body length and the monitoring month. Bottom right represents the range of body lengths (mm) for juvenile males and females in February (when monitoring for the species has historically been conducted) compared to those for adult males and females.

5.6. Discussion

5.6.1. Population structure

The *B. robustus* population composition showed loose synchronisation over the monitoring period such that adults and multiple different instars co-occurred in most months. Visible early instar nymph activity appeared to peak in late December, and most nymphs were large enough (> 8 mm) to be incorporated into removal sampling by January. Early instar nymphs continued to appear until late January indicating that hatching was not synchronous, however it did appear to be restricted to the mid-summer months. Variations in hatching times within a season could be due to subtle microclimatic and thermal differences where the eggs are laid (van Wingerden et al. 1991) because

nymph emergence is driven by thermal cues (Mason 1971). More broadly, White (1994) observed that *B. robustus* nymph emergence occurred earlier in warmer locations at lower altitudes within its range, compared to populations at higher elevations. The exact timing of nymph emergence is likely to show annual variation based on weather patterns. Juvenile instars were present throughout the entire monitoring period at various stages of development. Juveniles were largest in November and would have reached adulthood in subsequent weeks. Juveniles in November were larger than juveniles in March, supporting White (1994)'s suggestion that *B. robustus* over-winter as mid to late instar juveniles and reach adulthood in the following summer. This phenomenon occurs in other species of *Brachaspis* grasshoppers in New Zealand (Batcheler 1967, Hudson 1970, Ramsay 1978), and likely provides an adaptive advantage for persistence in an environment where temperature can be unpredictable all year around (Danks 1992). The prevalence of adults in the population was greatest in December and showed substantial decline in January, particularly for females. This decline likely reflects natural post-reproductive mortality, and any adults present after December were either late to reach maturity or long-lived relative to the majority. The more rapid decline of adult females compared to males could also reflect predation mortality if the female's larger body size results in them being more conspicuous to vertebrate predators (Tsurui et al. 2013).

5.6.2. Grasshopper distribution

The density of grasshoppers appeared to be patchy within and between plots over the sampling period, but patchiness could not be linked to ground cover. Plot A, which had the least bare gravel and most *R. australis* cover out of all the plots had grasshopper densities comparable to plots B, C, D and E. Similarly, plot F had substantially higher grasshopper densities despite having similar vegetative cover to plots B, C, D and E. A qualitative visual assessment of plot F revealed several factors that might have driven grasshopper densities to be higher at that location. Several metres beyond the northern edge of plot F the gravel habitat is replaced by a self-established grove of the woody weed *Rosa rubiginosa*. This grove is likely to be an unfavourable environment for the grasshoppers because it is shady, and the vegetation is moderately dense. It could form a partial vegetative barrier to grasshopper movement resulting in inflated grasshopper densities within the plot. In addition, the grassland borders of plot F were observed to be sparse compared to the other five plots and may not have acted as an effective barrier to grasshopper movement. Consequently, grasshoppers may have moved into the plot from the borders during the removal study and inflated population counts. Finally, although flooding events were rare and never persisted, it is possible that grasshoppers became

temporarily 'trapped' in plot F after persistent rainfall if the poor drainage in neighbouring plot E inhibited travel in the southbound direction.

The patchiness of grasshopper densities across plots within the same month might be driven by habitat structure and the corresponding microclimatic conditions that occur at the local scale, including humidity, wind exposure and solar radiation (Willott 1997, Gardiner and Dover 2008). If local scale microclimate is a strong driver of grasshopper distribution, then it should be considered when selecting the locations of monitoring plots within the wider riverbed range. The Patersons Terrace environment used in this study is relatively stable, and plot structure is likely to remain comparable over time. However, a natural braided river environment is dynamic, and flooding events rapidly change the substrate and vegetation structure: for example, in a single flooding event, boulders may be replaced by a sand bar, or established vegetation may be uprooted and washed away, and sections of previously favourable grasshopper habitat may become unfavourable. Therefore, fixed long-term monitoring plots may not be suitable, and instead plot site selection should occur such that natural changes in habitat structure caused by the dynamic nature of a braided river does not obscure detection of grasshopper population trends.

5.6.3. Detectability

Grasshopper detectability was most closely linked to body length. Adult and late instar female grasshoppers were the largest individuals in this study and were the most likely to be detected in the first search of an area compared to males or smaller instars of either sex. There was no evidence that observer experience increased the probability of detecting a grasshopper because probability of detection did not increase over time. On the contrary, the probability of detection was lowest in months January, February and March when observer experience was high. One explanation is that high grasshopper densities in these months increased the chance of immigration into the plot during a monitoring event, hence violating the assumption of population closure and deflating probabilities of detection. Another explanation is observer distraction. In January, February and March grasshoppers were small and present at high density, and it was not possible for the observer to keep track and capture multiple grasshoppers at the same time. This resulted in some individuals escaping capture and would have deflated detection probabilities. Finally, there were few large grasshoppers present after December which would also deflate the detection probabilities for those months.

Weather is also likely to affect detectability. Most insects have strict thermal ranges for optimal activity because they are ectothermic (Forsman 1999). Timing monitoring to fall within the thermal range for activity and during favourable weather conditions will enhance probability of

detection as a jump response from the grasshopper when it is disturbed is more likely (Forsman 1999). Although the exact thermal thresholds for *B. robustus* were not measured in this study, jump responses were common under the conditions the study took place (fine, warm conditions > 14 °C) suggesting these conditions are suitable guidelines for monitoring.

Habitat structure is another abiotic influence on detectability. Habitat features, such as vegetation structure and interstitial spaces can sometimes produce different detection probabilities (Bailey et al. 2004). Because Patersons Terrace has a unique habitat structure compared to other more natural braided river sites where *B. robustus* occur, it is not recommended that the exact detection probabilities generated in this study be transferred to populations beyond that at Patersons Terrace. However, the key observations from this study, 1) that adult and late instar females have high detection probabilities and 2) adult females are present in highest abundance in December, are likely to be transferable to other habitat types (e.g. open braided river habitat).

5.6.4. Recommendations for long-term monitoring design

The findings from this study lead to some key recommendations for monitoring populations of *B. robustus*. Firstly, monitoring to detect long-term trends in population size should be restricted to late instar and adult female grasshoppers. Large females had the highest probability of detection compared to any other demographic. Using counts of large females as an index of population size maximises observer reliability even in the absence of experience, therefore maximising confidence in trend detection. The number of adult female *B. robustus* present represents the reproductive demographic of the population. For other threatened species, particularly when juvenile mortality is high, the reproductive demographic is often used as an index of population size because it minimises random variation. For example, conservation goals for the threatened braided river bird species kakī (*Himantopus novaezelandiae*) refer to the number of adult breeding pairs (Pierce 1996). Measured femur length was used as an indicator of adulthood in this study. However, a more efficient strategy for future population monitoring would be visual assessment without handling the individual. For example, limiting counts to individuals which appear to be > 30 mm in length would allow for positive identification with low observer experience (Fraser 1999), and also be representative of the reproductive population because even if females have not yet reached maturity, they will do so within several weeks.

A second recommendation is that monitoring should take place in late November to early December. This is when the density of adult and late instar females (i.e. the current season breeding population) is greatest. There is likely to be some year-to-year variability in the timing of peak adult

female densities because development is driven by temperature and resource availability (Dempster 1963) which is strongly linked to weather. We recommend conducting several visits during the expected peak time to overcome this annual variation in peak adult abundance, and variation inherently introduced by imperfect detection.

A third recommendation is to interpret population trend from biennial comparisons of adult counts. In this study it was observed that two overlapping generations are present. Adults present in one summer are the parents of adults present two summers later, and opportunities to mate between generations appear to be few given that most adults die before the onset of winter and maturing of the next generation. Therefore, population trend based on adult female counts are only appropriate when compared between every second year. To achieve a good estimate of population trend it is recommended that data from at least the previous 6 years (3 generations) is utilised.

The short-term nature of this study means that further refinements are required to optimise the monitoring protocol for detecting population trend for *B. robustus*, and it is recommended that the monitoring design be adapted as the species becomes better studied. High priority avenues of future research include evaluating the most appropriate search method (e.g. transect counts or plot searches), the number of search areas (e.g. how many transects), the number of replicated searches at those areas required to overcome random variability (Dennis et al. 2010), and the optimal weather conditions under which to conduct searches.

5.7. Conclusions

Conservation outcomes can be maximised when management decisions are made based on high quality data that accurately represent population trends. This study has demonstrated that in the absence of pre-existing long-term datasets, removal sampling is effective for rapidly informing future long-term monitoring design for a threatened insect. In particular, removal sampling has been used to understand the seasonal population demographics of a little-studied species, identify a highly detectable subset of the population appropriate for use as an index of population size, and refining the timing of monitoring within the season to capture peak adult abundance. This provides an invaluable starting point for designing a long-term monitoring protocol.

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Chapter 6 Designing monitoring protocols to measure conservation benefits for a highly cryptic threatened grasshopper

6.1. Preface

Although ways to maximise detectability have been identified in the previous chapter, uncertainty remains around the most appropriate search method to use, and how many search replicates are required to overcome low detectability. It is also possible that the patchy distribution of the species (Fraser 1999) could cause further errors when estimating population size if searches are not adequately replicated in space. This chapter continues the development of monitoring protocols for *B. robustus*. Here, population density and distribution monitoring protocols that overcome detection and spatial errors are developed using data that has been collected across two habitat types over two to three consecutive years. Population density monitoring is essential for measuring the persistence and growth of a translocated population (Parker et al. 2013), and also for measuring any impact the translocation may have had on the depleted source populations. Species distribution monitoring is useful for detecting individuals that may have dispersed away from the receiving habitat during a translocation and could inform where the population has established. Both types of monitoring have important applications for species conservation management too. Species density monitoring is essential for evaluating the outcomes of any conservation management action (Lyons et al. 2008), and for detecting sudden or rapid population decline that might invoke conservation action (Block et al. 2001, Cook et al. 2016). Similarly, species distribution monitoring could invoke action if an absence of the species from sites within their range is correlated with a lack of management, or less intense management. The findings presented in this chapter are intended to be implemented as future monitoring protocols during a translocation, and as regular monitoring to inform *B. robustus* management across the species' range.

A version of this chapter has been submitted to *PLOS One*.

Designing monitoring protocols to measure conservation benefits for a highly cryptic threatened grasshopper

Schori, J.C.[1], Steeves, T.E.[1] and Murray, T.J.[2]

1. School of Biological Sciences, University of Canterbury
2. School of Forestry, University of Canterbury

6.2. Abstract

Species monitoring is prone to errors that can obscure real trends in population size or distribution. This is detrimental for threatened species conservation because managers often rely on current trends to make informed decisions about when and how to take action. Traditionally, populations of the Nationally Endangered grasshopper *Brachaspis robustus* have been monitored using a single transect searched once annually. The traditional monitoring strategy is likely to suffer from detection and spatial errors because individuals are highly cryptic and patchily distributed. There are also no currently established protocols for monitoring the distribution of *B. robustus* throughout its range. Here, optimal population density and distribution monitoring protocols for *B. robustus* are developed. A transect search method is recommended over a plot search method for population density monitoring because although there was no difference in population density estimates, a transect search is easier to implement in the field. November and early December is the most appropriate time to conduct monitoring, and > 20 transect replicates with at least four survey replicates each were required to detect a significant change in adult female population size with power > 0.8. Occupancy modelling was investigated as a distribution monitoring technique for *B. robustus* by estimating the probability of detection (p_g) in natural and modified habitats. Detection of grasshopper presence was found to be high (p_g > 0.6) using a 100 m^2 transect in both habitat types under optimal (no cloud) conditions, requiring a minimum of three visits per season to have confidence in trend detection. Replicating monitoring over a short time period was found to be essential for overcoming detection errors both in distribution and density monitoring for a rare and cryptic insect.

Keywords: *Brachaspis robustus*; robust grasshopper; population monitoring; occupancy modelling; conservation; threatened species; cryptic insect; Orthoptera.

6.3. Introduction

Population density monitoring of threatened insects is susceptible to errors that can lead to inaccurate population estimates (Yoccoz et al. 2001). For example, spatial errors can arise when surveys are not adequately replicated across space and therefore do not represent spatial variation in densities (Yoccoz et al. 2001). This is important to consider when monitoring insects that are patchily distributed within their habitat, which often occurs when the underlying resource distribution such as host plants or vegetative structures are not uniform (e.g. butterflies) (Harker and Shreeve 2008). Even when sampling is adequately replicated in space, it is still important that sampling is replicated in time to overcome detection errors such as false absences where an individual is present but not sighted by the observer (Dennis et al. 1999, Dennis et al. 2010). These errors can arise from crypsis (e.g. colouration that mimics the habitat or another species), elusive behaviour (e.g. refuge seeking, or freeze responses), species phenology (e.g. timing of egg or larvae phases, or peak adult abundance) and environmental influences over short time frames (e.g. low activity during cold weather, or activity that is correlated with time of day) (Harker and Shreeve 2008). For threatened species management, trend assessment is relied upon for both measuring the conservation benefit that an action has provided (Lyons et al. 2008), and for making decisions about when to invoke action (Block et al. 2001, Cook et al. 2016), therefore it is vital that monitoring produces accurate data about real population trends.

Several characteristics of the Nationally Endangered robust grasshopper *Brachaspis robustus,* (Stringer and Hitchmough 2012, Trewick et al. 2014) an insect endemic to the Mackenzie Basin of New Zealand, make monitoring populations of this species particularly prone to spatial and detection errors. The species is patchily distributed within its habitat (Fraser 1999), and individuals are highly visually cryptic, with colouration that is pale to dark grey or green-brown and mimics the rocky and silty substrates of their gravel habitat in braided rivers and associated terraces (Figure 6.1). To be detected, the grasshopper is usually required to jump in response to observer approach, but sometimes *B. robustus* responds by taking refuge underneath rocks or similar habitat features (White 1994). As a non-stridulating species, *B. robustus* is also acoustically cryptic. Although adult females (body length up to 36 mm) have higher detection probabilities than adult males (up to 17 mm long) or nymphs, the probability of detecting any individual is still less than 1 (Chapter 5).

Figure 6.1. Adult female robust grasshoppers (*Brachaspis robustus*; back) are two to three times larger than adult males (front). The colouration of both sexes mimics braided riverbed gravels.

Annual population monitoring of *B. robustus* has historically focused on a single population that is restricted to a narrow gravel road. Monitoring within this linear habitat has consisted of only a single transect searched once by two observers on a single day in February during fine weather (Te Manahuna/Twizel Department of Conservation, pers. comms.). However, transect searches may not be accurate if only conducted once each year because they can produce highly variable counts (Fraser 1999). Furthermore, February populations of *B. robustus* are mostly comprised of nymphs, which have been shown to have lower detection probabilities than the larger adults of the species (Chapter 5). A recent study provided several suggestions for improving population density monitoring for *B. robustus*, including that monitoring should use adult female grasshoppers, the most detectable group, as an indicator for population density to maximise both biological relevance and probability of detection, and that it should be conducted in December when adult density peaks (Chapter 5). There remains uncertainty around whether a transect search is the most appropriate monitoring protocol, or how many replicates in time and space are required to give adequate power to detect a significant change in population size over time because there is a lack of multi-season replicated monitoring data for this species.

There remains uncertainty on how to conduct regular monitoring at the landscape scale that tracks abundance and distribution of *B. robustus* across its full range to better inform conservation

management of this species. The species is known to be patchy within its range (White 1994, Fraser 1999), but the drivers of patchiness have not been well explored. The accepted distribution of *B. robustus* is based on maps created during bird surveys and other work > 25 years ago (Maloney 1992) supplemented with irregular partial surveys at a limited number of sites, and *ad hoc* observations since. In combination with the absence of recent targeted monitoring across the full range, these maps may not reflect the grasshoppers' current distribution because poor detectability could have resulted in false absences. In addition, there have been no reports of *B. robustus* in the southern-most extent of its range since the species was first discovered in 1960 (Bigelow 1967), indicating that range has contracted in the recent past (White 1994). Currently there are no protocols for monitoring *B. robustus* at the landscape scale. However, occupancy modelling might be a suitable monitoring protocol because it incorporates detection probabilities (MacKenzie et al. 2002), and can be used to both model species distribution (Karanth et al. 2011, Kery 2011) and to monitor the changes in distribution over time with respect to habitat degradation or conservation management action.

The aim of the current study is to develop optimal protocols for both population density, and population distribution monitoring of *B. robustus* that overcome spatial and detection errors. First, a transect search method is compared to a plot search method to determine which is the most appropriate for population density monitoring. Then a transect search protocol is applied in two different habitat types to determine the optimal number of transects, and search replicates required to detect a significant change in *B. robustus* population size over time. Finally, occupancy modelling is evaluated as a distribution monitoring protocol for *B. robustus* by estimating probabilities of detection. This study provides a valuable example of how monitoring protocols can be developed for cryptic threatened insects to improve data quality and maximise conservation outcomes.

6.4. Methods

6.4.1. Site descriptions

This study was conducted at two sites. The first, Patersons Terrace (Figure 6.2A), is an unused gravel road situated ~8 km SW of Tekapō that was laid during the construction of the Tekapō canal in the 1970s (McKay et al. 1978). It is not known whether *B. robustus* colonised this site naturally from the Forks River, or if a population was transported to the site with the gravel extracted from the Tekapō River for its construction. The road substrate is comprised of gravel (small stones < 64 mm diameter) and larger cobbles that have been compacted by historical heavy vehicle use. Vegetation is sparse and consists mostly of lichens and low stature vascular plants such as *Raoulia australis* and hawkweed

(*Hieracium* spp. and *Pilosella* spp.) (Chapter 5), as well as occasional *Rosa rubiginosa* and grasses. The gravel habitat is boarded by semi-modified grasslands dominated by fescue tussock and exotic pasture grass (Department of Conservation 2004) that extends for several tens of metres to the east before falling away as steep terraces to the Tekapō canal, and for several kilometres to the west. The grassland is unfavourable habitat and likely isolates the population from others present nearby (Chapter 3). The gravel road has been fenced off from regular vehicle use for several decades, however, it is open to introduced predatory mammals, and until recently domesticated sheep (*Ovis aries*) and introduced rabbits (*Oryctolagus cuniculus*) and hares (*Lepus europaeus*) grazed the grassland borders.

The second site, Snowy River (Figure 6.2B), is an alluvial fan with characteristics of a braided river that flows intermittently throughout the year. The river begins at the top of the valley between the Grampian Mountains and the Dalgety Range then flows west, becoming braided in the lower reaches as it crosses low-productivity farmland before draining into a large wetland. *Brachaspis robustus* are established on the lower braided section (~600 m wide). This is the eastern most population of *B. robustus* and is isolated from other populations by farmland and wetlands. Occasional flooding events, which are characteristic of braided rivers (Gray and Harding 2007), disturb the riverbed and remove vegetation. Mosses, lichens and herbaceous plants (e.g. Viper's bugloss, *Echium vulgare*), establish quickly after flooding events and larger, woody plants (e.g. *R. rubiginosa*) are present on stable sections of the riverbed. Substrate size is diverse and includes fine sands, cobbles and large boulders.

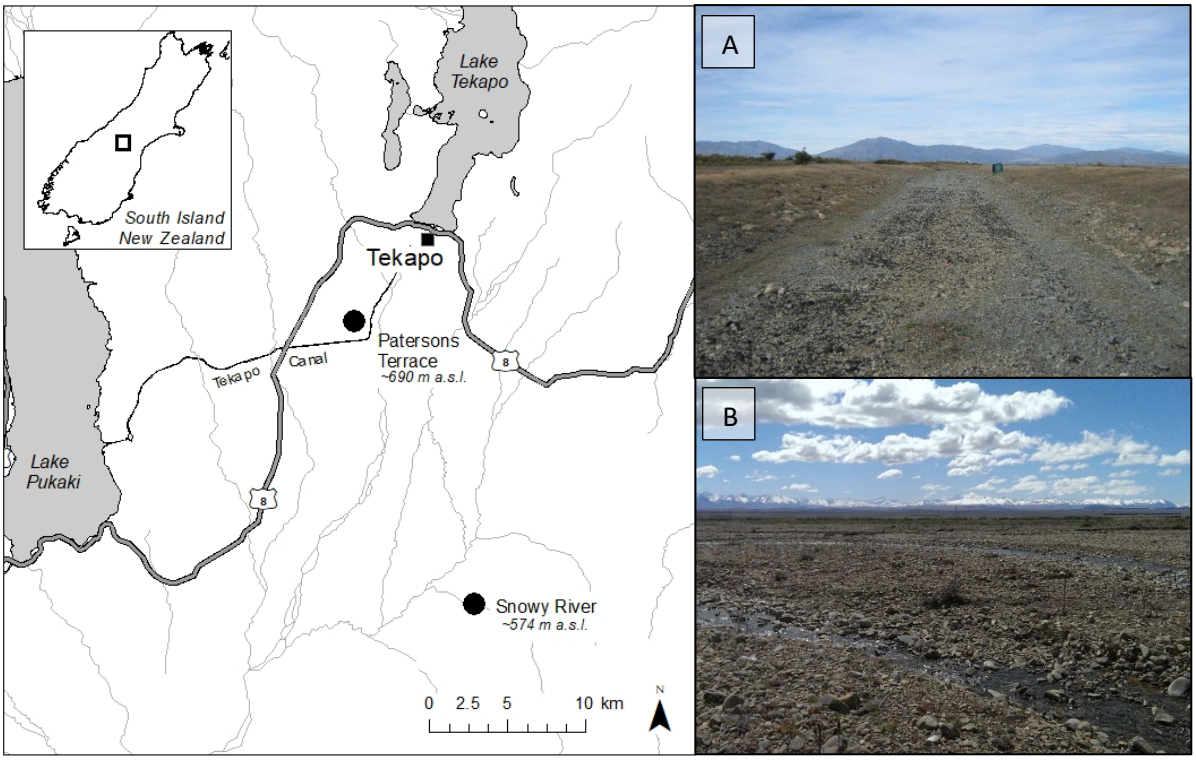

Figure 6.2. The location of Patersons Terrace and Snowy River in the Mackenzie Basin in the central South Island, New Zealand (inset). (A) Patersons Terrace is an unused gravel road. (B) Snowy River is an alluvial fan with braided river characteristics.

6.4.2. Field methods

Plot searches and transects searches of equal area were compared within the Patersons Terrace habitat during the summer (November to March) for three consecutive seasons (2015-16 to 2017-18). Three 100 m x 1 m (100 m^2) transects spaced ~1 km apart were set up along the centre of the gravel road. The transects were marked with flagging tape tied around metal pegs every 20 m, and numbered rocks every 1 m. Over each 100 m sampling stretch, four evenly spaced 5 m x 5 m (25 m^2) plots were defined on alternating edges of the road. Henceforth, a group of 4 plots (equal to 100 m^2) is referred to as a "plot unit". Flagging tape tied around a metal peg was used to the mark the corners of each plot. The plots and transects remained in the same location over the duration of the study.

Transect searches were conducted at Snowy River during the second (2016-17) and third (2017-18) seasons only. Three 100 m x 1 m transects spaced > 200 m apart were set up longitudinally along the riverbed in locations where grasshoppers were known to occur. Transects were marked with flagging tape tied around large yellow plastic pegs placed at 20 m intervals. In the third season, an additional two 100 m x 1 m transects were set up at the site. The three original transects were set up

as close as possible to their initial location, however changes in channel morphology resulted in minor deviations of less than 8 m.

Monitoring took place from November to March each summer on days of suitable weather (ground temperature > 14 °C and not during high winds or precipitation). Attempts were made to search each transect and plot unit on at least 6 days per month when feasible (Patersons Terrace, season 1 = 2 to 8 visits per month, season 2 = 4 to 8, season 3 = 6; Snowy River, season 2 = 3 to 7, season 3 = 6). All plots and transects were searched at both sites on the same day except where weather was not permitting (e.g. rain at one of the sites). Prior to commencing a search, barometric pressure, air temperature at 1 m above the ground, and ground surface temperature in the shade and sun were measured using a Kestrel 3500 Pocket Weather Metre (GeoSystems New Zealand Ltd). Cloud cover (categories: none; high cloud, when cloud was high in the sky but did not cause shadows; patchy cloud, when clouds were lower in the sky and caused shadows; overcast) and wind conditions (categories: none, low winds, high winds) and search duration were recorded for each search. During the search, the observer moved in a direction such that their shadow fell on the area already searched. The observer walked slowly across the entire defined area, sweeping the front foot over the ground to illicit a jump response from grasshoppers to make visual detection possible. When detected, grasshoppers were captured, their body length (from the top of the head to the tip of the abdomen) and femur length were measured, and sex and transect location were recorded. Each grasshopper was then released behind the observer to ensure it was not re-counted, and the remainder of the area was searched. Because accurate methods to determine when an individual had reached adulthood had not been developed at the time of this study, "adulthood" was based on measured femur length. Females were considered "adult" if their femur was ≥ 15 mm, and males were considered "adult" if their femur was ≥ 9 mm. The presence of juveniles with a body length < 8 mm was noted, but individuals were not captured because of the high risk of causing fatal injury to the grasshopper.

6.4.3. Data analyses

6.4.3.1. Population parameters

Population parameters were estimated using transect counts at both sites. Reproductive output for each site and generation was estimated under the assumption that eggs require exposure to cold winter temperatures to complete development (Chapter 2), therefore reproductive output was estimated by dividing the average number of juveniles per 100 m^2 in February by the average number of adult females per 100 m^2 in November and December in the previous summer (13- 15 months

prior). December was excluded from generation 2 at Patersons Terrace because no adult females were observed in that month. Survivorship of males and females were estimated for each site by dividing the average number of adults per 100 m^2 present in November and December by the average number of juveniles per 100 m^2 in February of the previous summer (same year). Estimates of total number of adult females present at both sites in November of each year of monitoring were generated by extrapolating mean counts per 100 m^2 over the area of suitable available habitat. Only November data were used for this estimate because of a high occurrence of zeros in data collected in December. For Patersons Terrace, this was estimated over 2.5 km of gravel road (17,501 m^2 habitat area), and for Snowy River, for 3 km of river (359,996 m^2 habitat area, Appendix C).

6.4.3.2. Search method comparisons (Transect versus Plot)

All analyses were conducted in *R* (R Development Core Team 2011) unless otherwise stated. For all three seasons of sampling at Patersons Terrace, total grasshopper counts from transect searches (three transects of 100 m x 1 m) and plot unit searches (three plot units comprised of four 5 m x 5 m plots) were pooled to give a count per day for each search method (over a combined 300 m^2 search area). The daily count generated from the two search methods was compared using a generalised linear model. A negative binomial distribution was fit using *MASS* (Venables and Ripley 2002) to account for overdispersion in the data. Model fit was checked by ensuring dispersion and Pearson's χ^2 was below the χ^2 5 % critical value. Search method, month, and season were specified as covariates. Model selection from nested candidate models was done by selecting for low AIC (Akaike Information Criterion). The mean daily count generated by the two methods was also compared for adult female *B. robustus* separately. Input data were limited to November and December when adult female abundance peaks. Season and month were considered as additional covariates in the Poisson model fit in *lme4* (Bates et al. 2015). Model fit was checked as above. ANOVA with a χ^2 was used to determine the significance of covariates, and model selection from nested candidate models was done by selecting for low AIC.

For seasons 2 and 3, the Index of Dispersion ($D = \sigma^2/\mu$) was used to calculate how much variability there was in the total population count generated by each sampling method (1 x 100 m^2 transect, or 4 x 25 m^2 plots) at Snowy River and Patersons Terrace within each month (November to March) and compared using nested ANOVA. Search method (categories: Patersons Terrace transects, Patersons Terrace plot searches, Snowy River transects) was specified as a fixed effect, and season and sampling unit were specified as random effects. Residuals were visually assessed for normality

and a slight left skew existed. Removal of outliers (D ≥ 4) improved normality of residuals but did not affect model output, therefore outliers were retained in the dataset analysed.

6.4.3.3. Detecting population trends

Power analyses (using *R* version 3.5.0) were used to determine the number of transects and survey replicates occurring on different days ('visits') that were required to detect a significant change in population size ($p < 0.05$) at Patersons Terrace and Snowy River with 0.8 power. The analyses were conducted on four datasets, adult female data collected throughout November and December at 1) Patersons Terrace, and 2) Snowy River, and full population (*B. robustus* of any age or sex) data collected in February at 3) Patersons Terrace, and 4) Snowy River, so that a new recommended monitoring regime could be compared to the historic protocol at both habitats. The count of grasshoppers detected on a transect during each visit was modelled using a generalised linear mixed effect model with a Poisson distribution in *lme4* (Bates et al. 2015). Year was modelled as a fixed effect and transect and visit were random effects. An additional parameter that accounted for random effects at the observation level was also considered. ANOVA and AIC were used to compare models that contained an observation level random effect to those that did not. The observation effect was only retained in the model if it explained a significant ($p < 0.05$) amount of variation. If there was no significant difference ($p \geq 0.05$) between the models, then the model with the lowest AIC score was selected. Using the model parameters, count data were predicted 1000 times for each combination of 25 visits and 40 transects. If a simulation produced an error, it was assumed that no significant difference could be detected, and p was set to 1. The power to detect significant (at $p < 0.05$) change in population size was calculated by dividing the number of simulations producing a significant p-value by the total number of simulations run.

6.4.3.4. Probability of detecting grasshopper presence

To inform an occupancy modelling design for *B. robustus,* the probability of detecting 1) a grasshopper of any age or sex, and 2) an adult female grasshopper on a 100 m x 1 m transect (compared to detecting zero grasshoppers) in an area known to be populated was estimated using generalised linear mixed effects model with a binomial distribution in *lme4* (Bates et al. 2015). Study site (categories: Snowy River, Patersons Terrace), month (categories: November, December, January, February, March), cloud cover (categories: no cloud, high cloud, patchy cloud, overcast) and ground temperature were considered as fixed effects in the model, and season and transect were specified as random

effects. Temperature did not have a significant effect and because of missing values, was excluded from further analyses and model selection. Model selection from nested models was conducted using ANOVA with a χ^2 test and selecting for lowest AIC. Model fit was assessed by visually checking for overdispersion. The analysis was repeated using a 20 m x 1 m resolution. Each 100 m^2 transect was divided into five 20 m^2 sections, and presence or absence of grasshopper detection within each segment was determined from the locations that were recorded when each grasshopper was captured. Pairwise comparisons were conducted in *lsmeans* (Lenth 2016), using a Tukey adjustment, and visualised using *multcompView* (Graves et al. 2015).

6.5. Results

6.5.1. Population parameters

Approximately four partial generations of grasshoppers were observed at Patersons Terrace across three monitoring seasons, and three partial generations at Snowy River across two sampling seasons. At both sites, hatching of nymphs occurred between November and January each year. Nymphs became large enough to incorporate into the study (i.e. > 8 mm body length) in January. After hatching, females lived for a further 12 – 14 months including overwintering. Grasshoppers were larger when monitoring recommenced in November than they were when monitoring ended in March indicating that growth continued during at least some of the months not monitored in this study (April – November). Once adults, females rarely persisted beyond December, however adult males were found in most months (Figure 6.3).

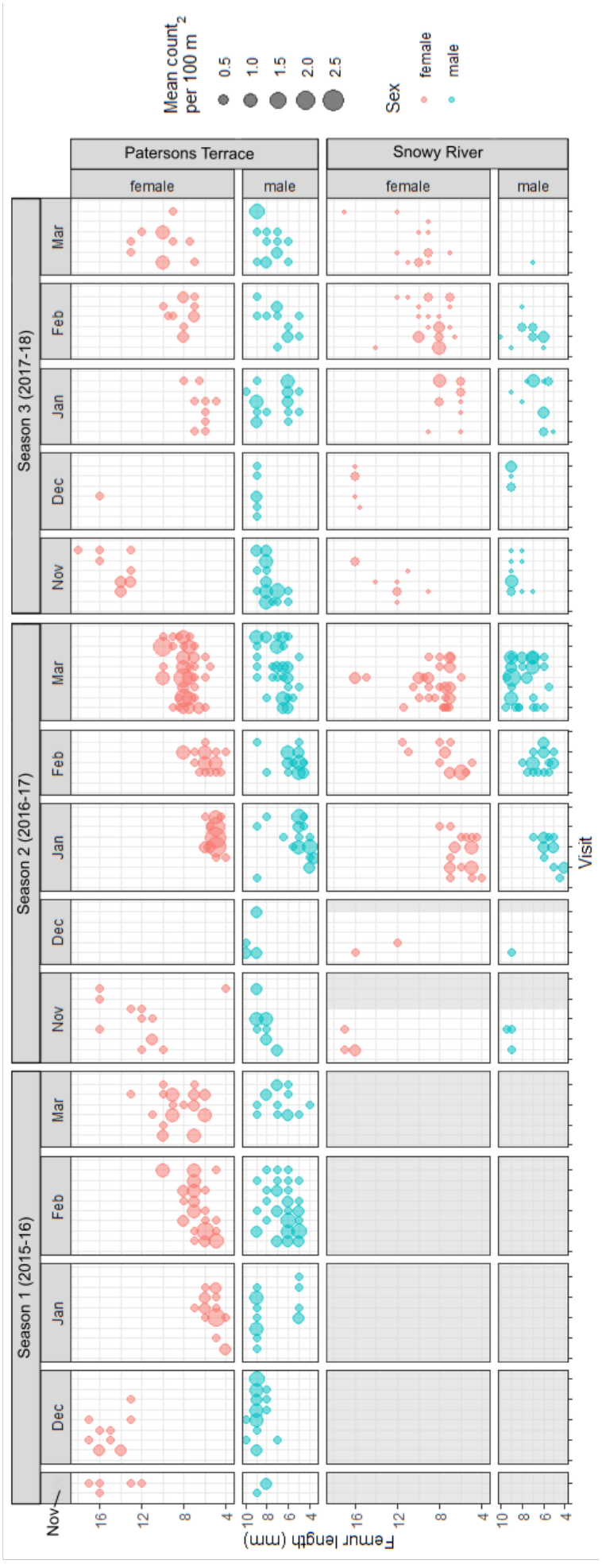

Figure 6.3. The mean number of grasshoppers per 100 m^2 (represented by dot size) by femur length (mm) estimated using 100 m x 1 m transects at Patersons Terrace (n = 3) and Snowy River (2016-17, n = 3; 2017-18, n = 5) during each monitoring visit. Each tick mark indicates a monitoring replicate ('visit') to a site. Grey shading indicates when monitoring visits did not occur. Males with femur length ≥ 9 mm and females with femur length ≥ 15 mm were considered 'adult' in this study. Juveniles of body length < 8 mm are not represented on this figure.

The highest number of adult females, adult males, and grasshoppers in total captured over a single 100 m^2 transect was 2, 3 and 16 at Patersons Terrace, and 3, 2 and 10 at Snowy River respectively. The observed population density of *B. robustus* was higher at Patersons Terrace than Snowy River in all months (Figure 6.4). However, when extrapolated over the area of available habitat, Snowy River is estimated to support a larger population (Figure 6.5). Male grasshoppers were estimated to have better survivorship to breeding age (4-22 % higher) than females at both sites, and reproductive output was estimated to be 22 % higher at Snowy River than Patersons Terrace for generation 2 (Table 6.1).

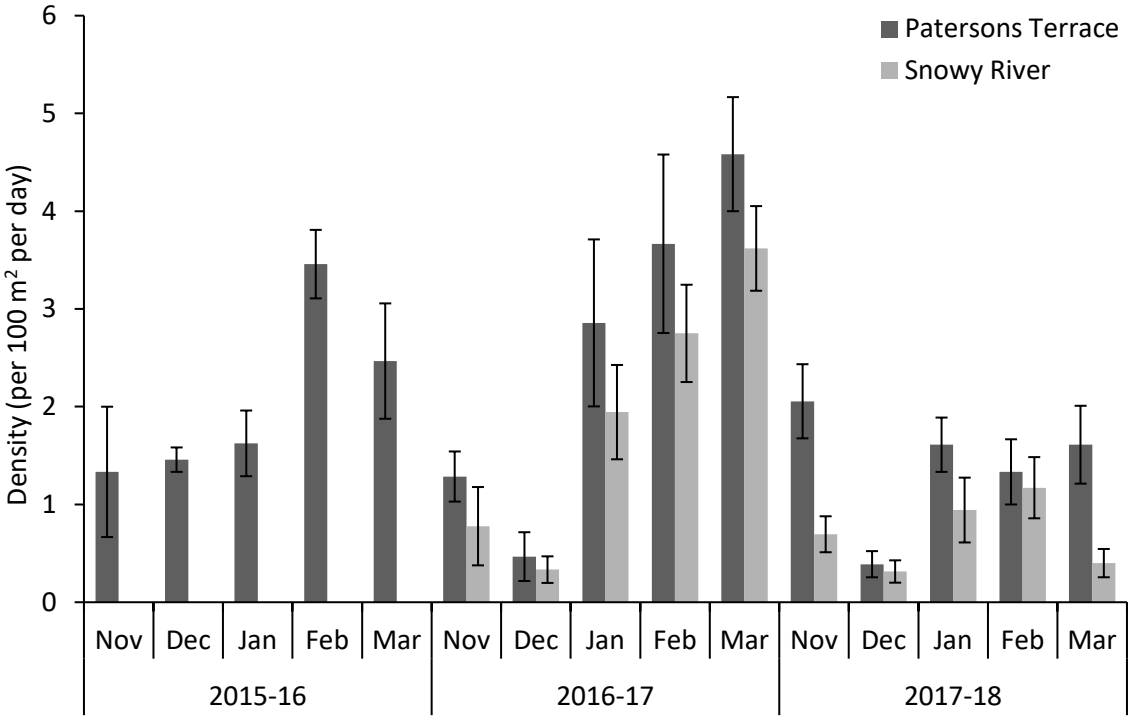

Figure 6.4. The mean (± SE) density of *B. robustus* (including adults and nymphs of both sexes) per 100 m^2 per day at Patersons Terrace and Snowy River during the monitoring period (November – March) for three seasons (2015-16 to 2017-18). No monitoring took place at Snowy River in 2015-16.

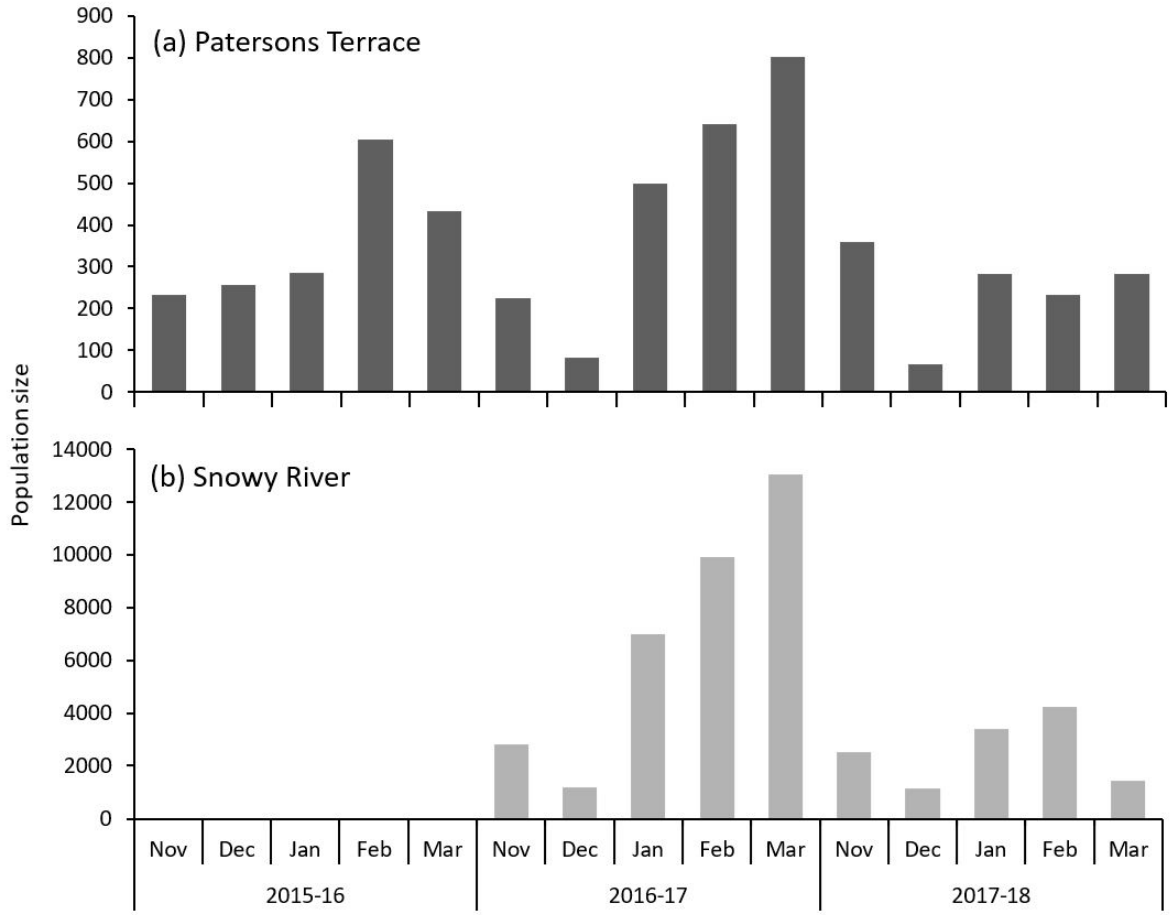

Figure 6.5. The estimated total number of *B. robustus* nymphs and adults present per month based on estimated area of available habitat at (A) Patersons Terrace and (B) Snowy River between November 2015 and March 2018.

Table 6.1. The estimated population parameters for *B. robustus* at Patersons Terrace and Snowy River between November 2015 and March 2018. 'Generation' refers to separate cohorts of grasshoppers reaching adulthood respectively in November and December of; 1 = 2015, 2 = 2016, 3 = 2017. 'Survivorship' is the estimated mean percentage of February nymphs that reach reproductive age, and 'reproductive output' is the estimated mean number of February nymphs per adult female.

	Generation	Patersons Terrace	Snowy River
Estimated habitat area		17,501 m^2	359,996 m^2
Reproductive output	1	10.75	
	2	8.56*	30.77
Female survivorship	2	8.36%	
	3	9.09%	10 %
Male survivorship	2	21.62%	
	3	12.70%	32 %
Estimated number of adult females in population (November)	1	88 (*n* = 2)	
	2	25 (*n* = 7)	1600 (*n* = 3)
	3	30 (*n* = 6)	239 (*n* = 6)
Estimated adult female density per 100 m^2 (November)	1	0.5 (*n* = 2)	
	2	0.14 (*n* = 7)	0.44 (*n* = 3)
	3	0.17 (*n* = 6)	0.07 (*n* = 6)

n = number of visits made to that site in the month
* December 2016 data excluded in calculation because no adult females were detected
Grey shading indicates data not available because Snowy River was not monitored during season 1

6.5.2. Search method comparisons

Search time for plots and transects was higher when more grasshoppers were present due to the time spent capturing and processing individuals. The minimum time required to search a 100 m^2 transect was 5 mins, and for each plot was ~1.5 mins (~6 mins per 100 m^2 plot unit including time taken to walk between plots). There was no significant difference in the Index of Dispersion for data collected using plot searches or transect searches at either site (d.f. = 2, 92.2, F = 1.71, p = 0.19). Population counts from transects were on average 8 % (± 9 % SE) lower than counts from plots, but the difference was non-significant (p = 0.32). Compared to November, counts from December did not differ significantly (p = 0.67) but counts in January (p < 0.001), February (p = 0.02) and March (p = 0.004) were significantly higher by 84 %, 43 % and 52 % respectively. Season 1 and 2 counts were not significantly different (p

= 0.23), but counts in season 3 were on average 40 % (± 11 % SE, $p < 0.001$) lower than season 1 (Figure 6.6).

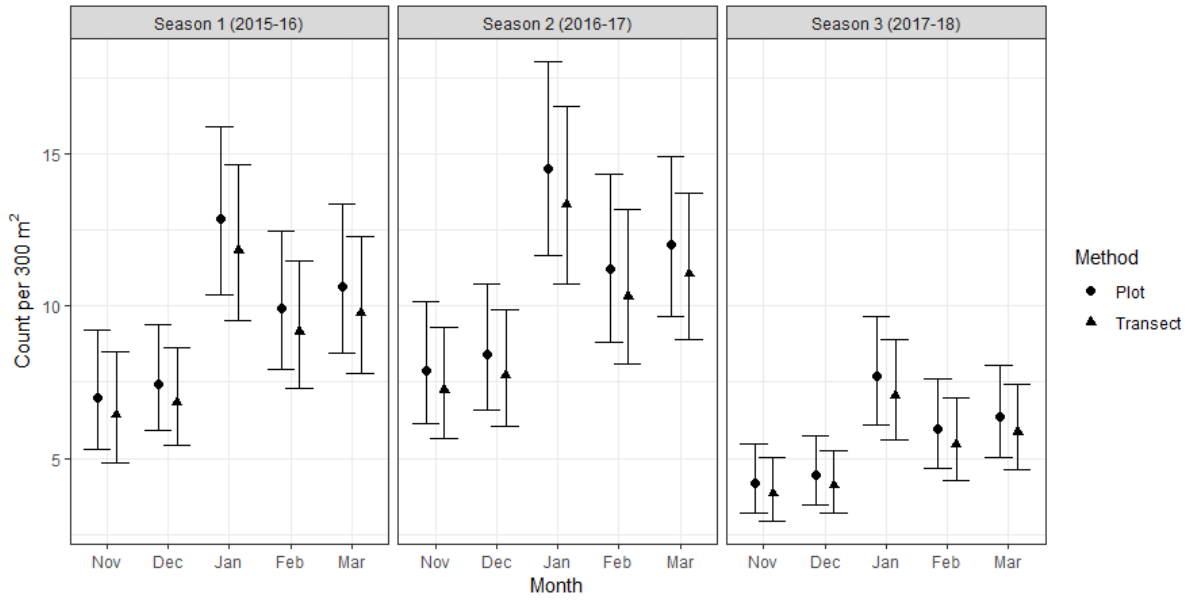

Figure 6.6. The predicted mean number of *B. robustus* grasshoppers within the 300 m^2 sampling area using plot searches (12 plots x 25 m^2) and transect searches (3 transects x 100 m^2) at Patersons Terrace during the monitoring period (November – March) for three seasons (2015-16 to 2017-18).

6.5.2.1. Adult female grasshoppers

On average, searches using transects detected 6 % (± 40 % SE) fewer adult females than plots, but the difference was non-significant ($p = 0.87$). Counts did not significantly differ between November and December ($p = 0.14$), but on average 41 % (± 44 % SE) fewer females were detected in December. There were significantly fewer adult females detected on average in both season 2 (-55 %, $p = 0.046$) and 3 (-80 %, $p = 0.002$) compared to season 1 (Figure 6.7).

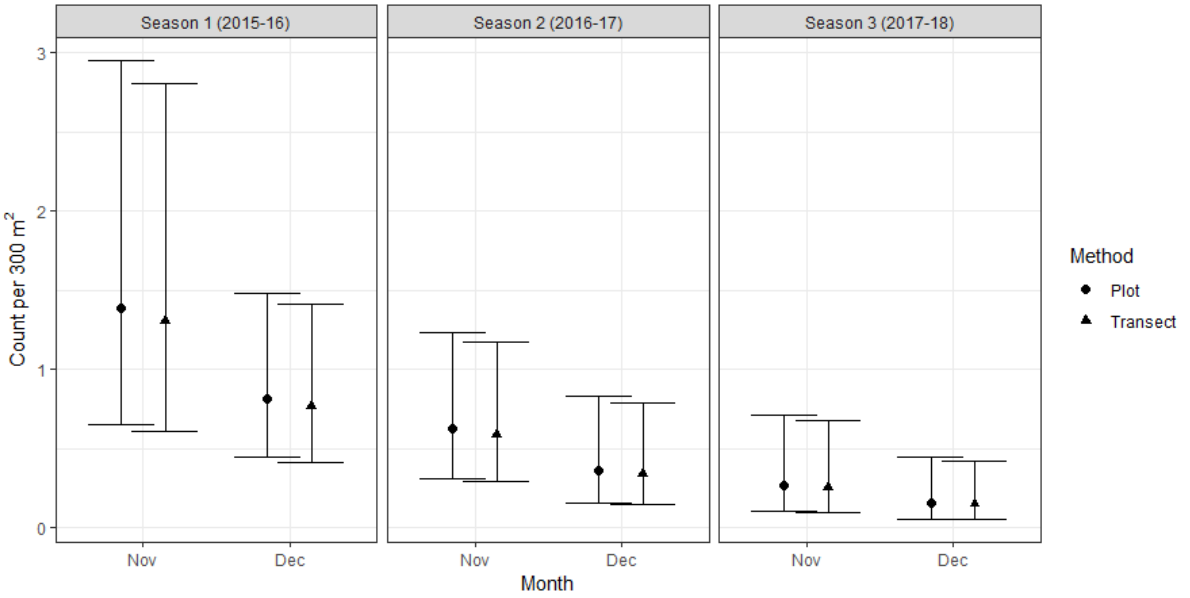

Figure 6.7. The mean number of adult female *B. robustus* grasshoppers detected in 300 m^2 of sampling area using plot searches (12 plots x 25 m^2) and transect searches (3 transects x 100 m^2) at Patersons Terrace during peak adult occurrence (November and December) for three seasons (2015-16 to 2017-18).

6.5.3. Detecting population trends

The power to detect a significant change ($p < 0.05$) in population size at Snowy River when monitoring adult females in November and December was low for all combinations of transect counts and survey replicates modelled. At Patersons Terrace, power of 0.8 could be achieved using a minimum of 7 transects and 10 replicate surveys, or 20 transects and 4 replicates. Power was much higher for both sites when observing the full population in February (Figure 6.8).

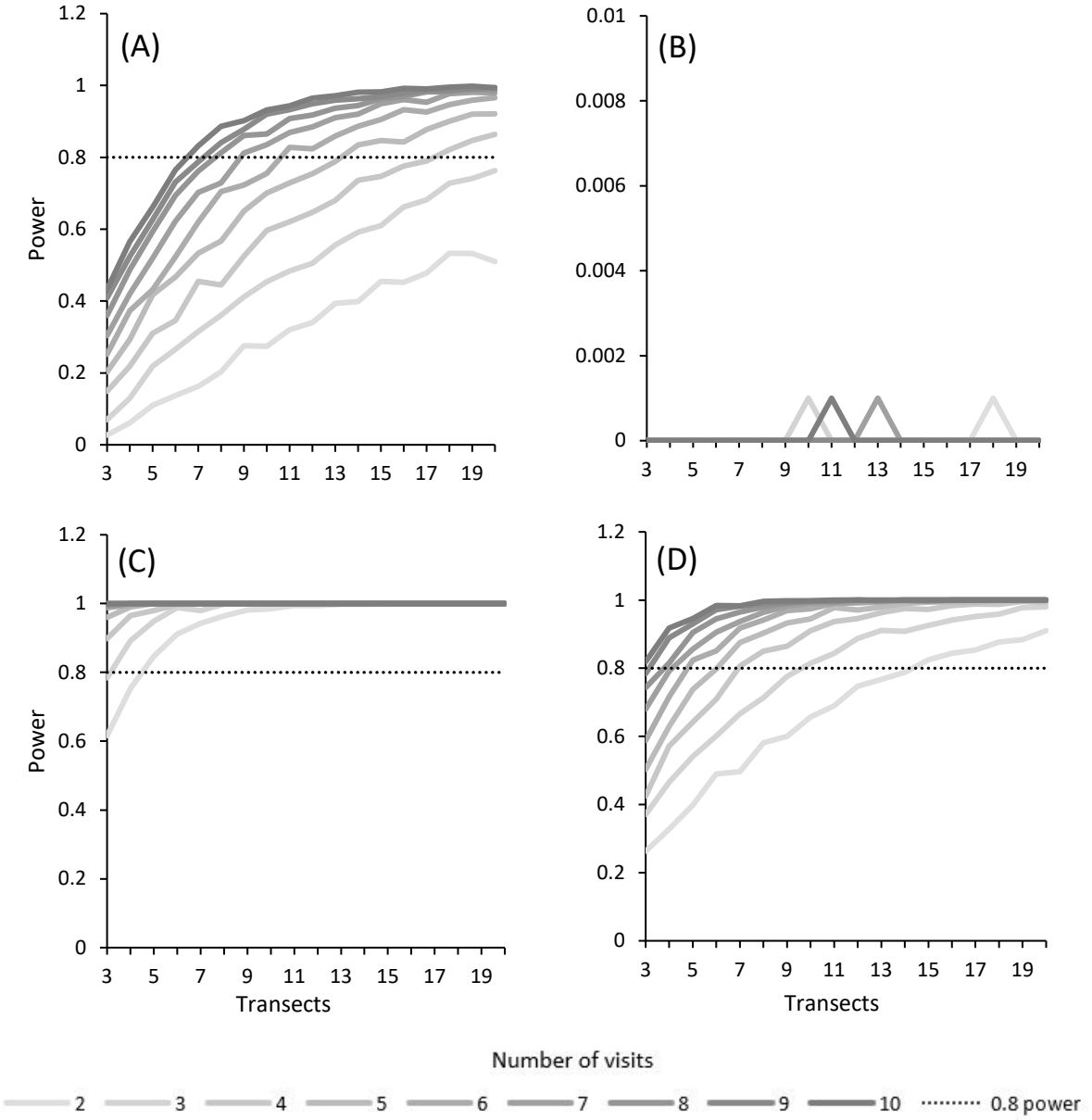

Figure 6.8. The power to detect a significant ($p < 0.05$) change in *B. robustus* population size with increasing number of transects and survey replicates ('visits') using adult female data collected in November and December at (A) Patersons Terrace, and (B) Snowy River, and total population (any age or sex) data collected in February (historical monitoring methods for *B. robustus*) at (C) Patersons Terrace, and (D) Snowy River.

6.5.4. Probability of detecting grasshopper presence

Detection probabilities were generally lower using 20 m x 1 m transects (20 m^2 search area) compared to 100 m x 1 m transects (100 m^2 search area), but showed similar trends with respect to site, cloud cover and month (Figure 6.9). Holding month and cloud cover constant, the probability of detecting a grasshopper (p_g) was on average lower at Snowy River than at Patersons Terrace (20 m^2, 55 % lower,

p = 0.001; 100 m^2, 53 % lower, p = 0.003). The probability of detecting a grasshopper was highest and less variable under 'no cloud' conditions (20 m^2, p_g = 0.32; 100 m^2, p_g = 0.88) and was lowest when 'overcast' (20 m^2, p_g = 0.12; 100 m^2, p_g = 0.42), and was higher in January (20 m^2, p_g = 0.40; 100 m^2, p_g = 0.89) and February (20 m^2, p_g = 0.41; 100 m^2, p_g = 0.94) than for any other month (November, December, March). Pairwise comparisons are presented in Appendix D. The probability of detecting an adult female *B. robustus* was less than 0.15 at both Patersons Terrace and Snowy River for both 100 m^2 and 20 m^2 transect lengths (Figure 6.10).

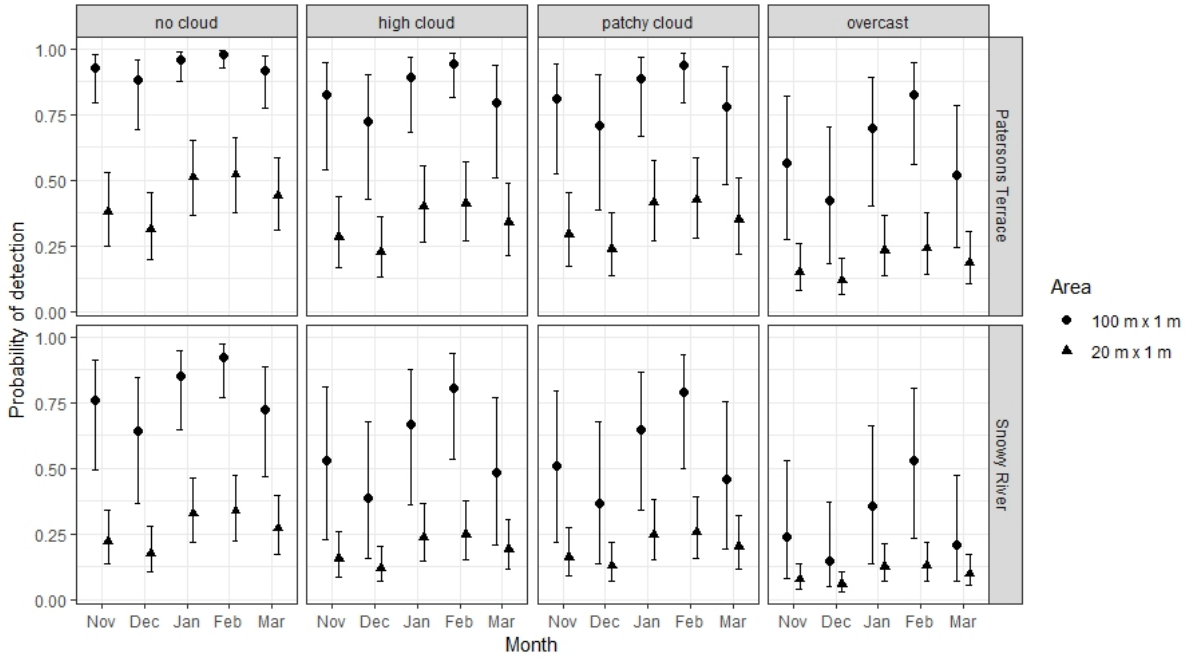

Figure 6.9. The probability (± SE) of detecting a *B. robustus* grasshopper on a 100 m x 1 m (100 m^2) and 20 m x 1 m (20 m^2) transect at Patersons Terrace and Snowy River under four different cloud conditions: no cloud, high cloud, patchy cloud and overcast.

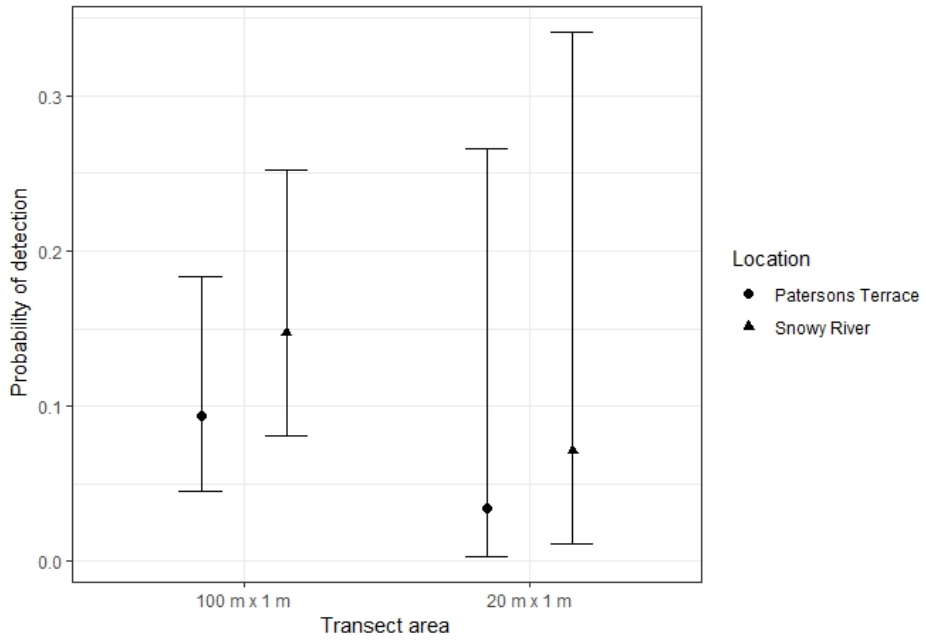

Figure 6.10. The probability (± SE) of detecting an adult female *B. robustus* along a 100 m x 1 m (100 m^2) and 20 m x 1 m (20 m^2) transect at Patersons Terrace and Snowy River in November or December.

6.6. Discussion

Distinct generations of female grasshoppers were observed throughout the monitoring seasons at both sites. Grasshoppers hatched in early summer and females reached adulthood approximately 12-14 months later but rarely persisted beyond December the following year. Female femur length was longer in November than in March of the same year indicating that some growth takes place over the winter period when monitoring for this study ceased. Adult males were found in most months making generations more difficult to distinguish. It was unclear whether adult males present after December were long-lived individuals that hatched the previous season, or if they were from the new generation that had matured early. In general, it appears that generational overlap is minimal and thus the opportunity for mating to occur between individuals from different generations is rare.

Population densities over 300 m^2 at Patersons Terrace did not differ between season 1 and 2 but were significantly lower in season 3. The lower density of mature generation 3 adults present in November and December of season 3 is likely to be explained by poor survivorship. Although female survivorship of generation 3 was higher than generation 2, male survivorship of generation 3 was almost half of generation 2 (Table 6.1). In addition, the lower density of generation 4 nymphs present in January to March of season 3 is likely to be explained by low reproductive output of generation 2

adult females. However, because population size at Snowy River was also lower in season 3 compared to season 2 (Figure 6.5), it is possible that there was a region-wide seasonal influence. Populations of Orthoptera can fluctuate rapidly in response to temperature (Willott and Hassall 1998) and food availability (Dempster 1963) meaning other seasonally variable abiotic factors, such as the number of frost days and sunlight hours (Dempster 1963) that were not measured in this study may have had an effect. Research directed toward incorporating these parameters into a model that could be used to accurately predict population trends for *B. robustus* could benefit conservation management. For example, such a model could be used to prevent or minimise the severity of future *B. robustus* population declines if it triggers mitigation action such as captive breeding in years where survival or reproductive output is predicted to be low.

6.6.1. Transect versus plot monitoring

Grasshopper counts generated from plot searches and transect searches at Patersons Terrace did not significantly differ. This indicates that both search methods are appropriate for overcoming the patchiness of grasshopper distribution in space. However, it might be expected that the distribution of grasshoppers at Patersons Terrace is less patchy than would occur in an open braided river environment where the habitat is much more expansive, and less uniform. Given that transects had 202 m of exposed edge per 100 m^2 search area, compared to 80 m for plots, immigration and emigration rates were expected to be different for the two search types. However, the quick search time for both methods would have minimised any effect. The two search methods were also expected to be different because during a plot search an observer spends more time within a grasshopper's jump range, and the observer's search direction loops back and forth within the plot. It would be expected that more grasshoppers are double-counted using the plot method than the transect method where grasshoppers were released behind the observer. This effect may have been minimised here because the plots were small in area so the observer could keep track of individual grasshoppers to avoid double-counting. In larger plots double-counting could be more common. In general, both search methods are effective for monitoring grasshoppers when environments are homogenous, and immigration and emigration during the counting period is minimal (Gardiner et al. 2005).

Given little difference in counts or search time were found between plot and transect search methods in this study, a transect method is recommended for monitoring *B. robustus* populations because it is more practical to implement in a braided river environment. The 20 m spacing of static markers used to define transects in Snowy River was found to be sufficient for observer navigation and repeating the transects for two consecutive years was straightforward using GPS waypoints to re-

position the markers. Although the location of the transects did have to shift year-to-year to account for changes in channel morphology over winter, it was simple to establish new transects at close proximity to their previous location. It is likely that flexibility in transect locations over time to reflect changes in channel morphology is beneficial for monitoring *B. robustus*, because their distribution in the riverbed is likely to shift accordingly as well. In this study there were no instances where the transect path needed to change within a monitoring season (November to March). Although there was one instance of a major flooding event at Snowy River in February 2018 (Appendix B), the water quickly resided, and monitoring was able to resume along the same transect line. Another reason for recommending the transect method is because when implementing monitoring over a larger area it is likely that an observer will use a GPS to navigate rather than static markers. Under these conditions, a transect search method requires an observer to navigate from the start to the end of a transect whereas a plot search method would require navigation back and forth multiple times across a plot without crossing the same path, which is much more difficult to achieve. Finally, there is a lower chance of double-counting grasshopper using a transect search method than a plot search method for reasons discussed above.

6.6.2. Future long-term population monitoring design

This study provided evidence that a transect search method is appropriate for detecting changes in population size over time and confirmed that searches need to be replicated to over-come spatial and detection error and be able to detect significant changes in population size over time. The importance of replication was highlighted by several density and population size estimates. Density estimates for adult females in November at Patersons Terrace were similar in 2016 and 2017 when searches were replicated ≥ 6 times, however, when only 2 replicates were performed in 2015, the estimate was ~3 times higher. Similarly, at Snowy River density estimates of the same demographic were ~6 times higher when only 3 visits were made compared to when 6 visits were made. Although an underlying annual effect on the population size is expected to be present, these major discrepancies are most likely to arise from spatial or detection variability which is more prevalent when fewer replicated searches are undertaken (Dennis et al. 2010). The consequences of this variation can lead to biased population estimates, as seems to have occurred at Snowy River for adult females in generation 2. Inaccurate population estimates could have implications for species management if they obscure real population trends, and therefore impede informed decision making about when and what management action needs to occur.

There was high power to detect significant change in population size for monitoring that was conducted in February even when there few replicates in time or space. However, there are several reasons why February counts are not recommended for monitoring *B. robustus*. Individuals in February are small, with poor detectability (Chapter 5) and it may be difficult for inexperienced observers to distinguish them from other species (Fraser 1999). Most of the population in February are nymphs that are vulnerable to spontaneous fluctuations driven by environmental factors (Dempster 1963). Additionally, the timing of hatch within the season is driven by temperature (Dempster 1963, Mason 1971), and early or late hatching could contribute to annual variation in the number of February grasshoppers counted. Therefore, significant changes in population size might be easy to detect, but they do not contribute information about true population trends.

Previous research had suggested that transects need to be visited 10 times per season to improve statistical precision of counts for *B. robustus* (Fraser 1999). Our findings suggest that monitoring of adult females in November and December when using 20 transects requires 4 visits per season to have sufficient power (> 0.8) to detect a significant ($p < 0.05$) change in population size at Patersons Terrace. At Snowy River, power to detect change was low possibly because our simulation was run from only 2 seasons of data that contained a high proportion of 0 counts and limited the information available for conducting power analyses. We recommend that monitoring in natural braided river sites includes more search replicates than at Patersons Terrace to account for greater substrate heterogeneity which potentially leads to lower detectability. We also recommend that monitoring would optimally be limited to November to avoid low December counts that occur in some years, possibly because of early maturity and post-reproductive mortality that may be driven by early spring emergence.

6.6.3. Distribution monitoring using occupancy modelling

The probability of detecting an adult female at either study site was always low, however the probability of detecting any grasshopper (p_g) was higher at Patersons Terrace than Snowy River at both the 100 m x 1 m and the 20 m x 1 m search resolution. This is most likely to be because the density of grasshoppers was higher at Patersons Terrace than at Snowy River, and could also explain why p_g was higher in the months following nymph emergence despite juveniles having lower individual detection probabilities (Chapter 5). Notwithstanding the fact that the habitat is much narrower at Patersons Terrace than at Snowy River, p_g could also be higher at Patersons Terrace because the substrate is small, uniform and highly compacted, and vegetation is low in stature, meaning there are fewer refuges for grasshoppers to retreat into when disturbed by an observer. In contrast, Snowy

River has diverse substrate size and seasonal riverbed disturbance creating interstitial spaces, as well as a higher diversity of vegetation stature where leafy vegetation could conceal grasshoppers. Cloud cover also had a significant effect on the probability of detection and is likely related to grasshopper activity. Grasshoppers are ectothermic, and basking is required to raise body temperature and increase activity (Forsman 1999). Because a jump response to observer disturbance is usually required for detection to occur, monitoring that takes place on fine warm days is likely to yield higher detection probabilities.

This study indicates that an occupancy monitoring design could be successfully implemented to monitor the distribution of *B. robutus* at the landscape scale. In general, when the probability of detection is high fewer visits are required to a site to have sufficient confidence in the presence or absence of the species (Mackenzie and Royle 2005). Low p_g was yielded in both sites when using a 20 m^2 transect, but a high p_g (> 0.6) was achieved at both sites using a 100 m^2 transect under optimal (no cloud) conditions. Mackenzie and Royle (2005) recommend that a minimum of three visits be made when p_g > 0.5. However, the Patersons Terrace and Snowy River populations of *B. robustus* are expected to be the densest (Morris 2005), and largest (White 1994), across the species range respectively, so p_g is expected to be lower at other sites. We recommend that to monitor *B. robustus* distribution at a landscape scale, a minimum of 3 visits be made per season to transects ≥ 100 m in length during fine warm weather, and that visits occur in February when probabilities of detecting grasshoppers peak.

6.7. Conclusions

This study has identified that replicated transect searches are an appropriate population density monitoring method for *B. robustus*. This study also identified that occupancy modelling is suitable for monitoring changes in *B. robustus* distribution across the range of the species. It has highlighted the importance of replicating monitoring searches in space and in time for small, cryptic and patchily distributed threatened insects, to ensure that monitoring data has enough power (> 0.8) to detect significant ($p < 0.05$) changes in population size. Investing time into producing monitoring protocols that detect accurate changes in population size or distribution over time, such as has taken place in this study, will benefit the conservation management of the species by ensuring managers are best informed when making critical decisions.

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Chapter 7 Discussion

The research presented in this thesis has generated knowledge required for developing conservation translocation into a successful management tool for threatened insects. Four key knowledge gaps in translocation design were investigated for the study species, *B. robustus*, a Nationally Endangered grasshopper endemic to the Mackenzie Basin of New Zealand. First, this study identified life history parameters of the species to better inform management. Second, important habitat requirements of *B. robustus* were identified to improve site selection and habitat management. Third, the appropriate level of mammalian predator control required within a receiving habitat to maximise translocation success for *B. robustus* was determined. Fourth, effective methods for monitoring the outcome of a translocation and measuring conservation benefits were developed. Here, the key findings for each knowledge gap are discussed in the context of improving conservation translocation success for *B. robustus* and advancing conservation management of threatened insects globally.

7.1. Life history

A thorough understanding of a species' life history traits and biological requirements for growth and reproduction is critical for achieving conservation translocation success for threatened insects. In Chapter 2 of this thesis, life history traits of *B. robustus* were investigated using captive rearing *in situ* and *ex situ* in the laboratory. A key finding was that *B. robustus* does not produce eggs until ~1 year after hatching, and those eggs hatch ~1 year after being laid. In a translocation context, this finding means that if juveniles comprise the founding population, an F2 generation at a release site would not be present until 2 years after release. This explains the continual population decline seen in the first 12 months of the initial experimental translocation to the kakī aviary complex in 2015 (Figure 4.3) and why no recruitment was observed 1 year after release. The most successful translocation release strategy for *B. robustus* is likely to require > 2 releases occurring in consecutive years. This will ensure an adult population is present each summer, rather than every second summer, and increase population resilience against stochastic or extreme events, as well as provide 'insurance' for seasons when conditions are less optimal for reproductive output.

In addition to establishing life history parameters, another purpose of Chapter 2 was to determine biological requirements for growth, development and reproduction and protocols for captive rearing. Captive rearing has the potential to be an effective conservation management strategy for insects because they generally have high reproductive potential, small body size, and high

levels of natural mortality before reaching sexual maturity (Dempster 1963). Rearing insects in a captive environment where common sources of mortality (e.g. predation) are absent, and resources are abundant could rapidly increase numbers of individuals. Furthermore, because insects are small, captive rearing requires less space and is less labour intensive when compared to birds or mammals (Morton 1983, Pearce-Kelly et al. 1998). Potential conservation applications include captive rearing individuals to augment wild populations, or to produce founder populations for translocations.

One concern is that adaptation can occur relatively quickly in captive populations of insects (Hoffmann and Ross 2018), and in some situations can be detrimental to achieving conservation success. Because laboratory environments usually provide optimal conditions that maximise growth and reproductive output they can also reduce selective pressures for resilience to stressors that would be experienced in the wild, e.g. disease, thermal extremes, desiccation and starvation (Hoffmann et al. 2001, Hoffmann and Ross 2018). Laboratory environments can also be selective for traits that reduce reproductive output or fitness in the wild. For example, captive rearing of Puget blue butterflies (*Icaricia icarioides blackmorei*) for a single generation caused wingspans to be smaller, and body length to be shorter than the wild stock populations, traits that are associated with lower fecundity and poorer dispersal ability (Schultz et al. 2009). These negative consequences can hamper conservation translocation success which relies on released individuals to mate, forage and reproduce effectively (Hoffmann and Ross 2018), but are usually successfully overcome in captive rearing programmes. For example, Schultz et al. (2009) recommended overwintering butterflies in outdoor enclosures to maximise selection for traits favouring survival in outdoor conditions. Outdoor overwintering would also be useful for overcoming some complex life history traits, such as the thermal cues required for breaking egg diapause in *B. robustus* (Chapter 2), that have not yet been experimentally determined or effectively replicated in a laboratory environment. For *B. robustus* it could be that an optimal captive rearing strategy incorporates a combination of the laboratory and field methods presented in Chapter 2 to provide conditions that minimise juvenile mortality (e.g. freedom from predators and extreme weather events), maximise reproductive output (e.g. warm temperatures and abundant food), and optimise hatching success (e.g. correct thermal requirements for egg development).

7.2. Habitat

Identifying suitable receiving habitat is important for conservation translocation success because it minimises dispersal upon release (Armstrong and Seddon 2008), and provides adequate resources for reproduction and population growth over time. Comparing *B. robustus* movements in a natural and

modified landscape in Chapter 3 provided several key insights that could improve translocation success and inform conservation management for *B. robustus* and other threatened insect species.

A key finding from Chapter 3 was that home range size of adult female *B. robustus* did not significantly differ between an open braided river habitat and a modified linear gravel road. This suggests that adult females require a minimum receiving habitat area of 250 m^2 to 300 m^2, regardless of habitat shape. Because the density at which *B. robustus* thrive is not known, it is unclear what carrying capacity relative to habitat size is, or what density is required to optimise mate finding. Given that the goal of a translocation is for a population to establish and grow over time (IUCN 2013), it is important that the receiving habitat for any species, including *B. robustus,* is larger than the minimum area required by the founders. However, home range measured at source habitat, which is often degraded or low-quality habitat, is not always a suitable indicator of optimal habitat size, dispersal behaviour, population density or range expansion of the species at the release site. For example, Cook Strait giant wētā (*Deinacrida rugosa*) travelled substantially further when released into Karori Wildlife Sanctuary compared to individuals at the source population on Mana Island (Watts et al. 2008). Similarly, Wellington tree wētā (*Hemideina crassidens*) established higher population densities at distances further away from the release site than *D. rugosa* following translocation to Matiu-Somes Island, despite being considered the less mobile of the two species (Watts et al. 2009). These examples indicate that although estimating home range size and movement distances of individuals in familiar territory can provide a valuable starting estimate of optimal habitat size, monitoring conducted after a translocation is the best indicator of post-release range expansion to inform future translocation habitat requirements.

Another key finding from Chapter 3 was that dense vegetation is unfavourable habitat for *B. robustus*. Translocation success could potentially be improved by utilising dense vegetation as a barrier to prevent dispersal upon release. Dispersal is common after a translocation, but can be detrimental to success because it further reduces the founder population (Armstrong and Seddon 2008). Although using vegetation to prevent dispersal is an option, many vegetative species are introduced and negatively affect braided riverbed dynamics (e.g. crack willow, *Salix fragilis*; broom, *Cytisus scoparius*; Russell lupin, *Lupinus polyphyllus* cultivar) and remove open gravel habitat that is required by many riverbed species, including *B. robustus* and threatened braided river birds (O'Donnell et al. 2016). A more appropriate approach could be to translocate *B. robustus* onto braid islands and use flowing river water as a natural barrier to dispersal instead. Using a flowing water border could also reduce mammalian predation pressure when compared to a vegetative border. Vegetation provides refugia for predatory mammals and could inflate their abundance in the translocation vicinity (Pascoe 1995). Furthermore, introduced predatory mammals are present at a

lower abundance on braid islands compared to the mainland (Pascoe 1995). Using islands as a translocation receiving habitat to isolate vulnerable populations from predatory mammals is a common approach in New Zealand (Towns and Ferreira 2001, Watts et al. 2008, Sherley et al. 2010, Miskelly and Powlesland 2013). However, a key risk of translocating *B. robustus* to a river island is flooding, which could potentially decimate a small, isolated population within a few hours. Translocating to large river islands or to multiple islands across several different rivers is recommended to minimise the risk of translocation failure from severe flooding events.

That dense vegetation is unfavourable habitat for *B. robustus* also highlights the importance of vegetation management for maintaining habitat quality and connectivity among individuals (i.e. prevent isolation from potential mates) at translocation receiving sites and wild sites. Following the construction of the Waitaki hydro scheme, some of the rivers that *B. robustus* currently inhabit no longer undergo severe flooding, an important natural disturbance event that removes vegetation from river banks and braids and forms exposed, rocky habitats (Tockner et al. 2006). Without floods, open exposed gravel is becoming increasingly vegetated resulting in habitat loss for *B. robustus* and other bare gravel habitat specialists (O'Donnell et al. 2016). Habitat loss from weed encroachment is also expected to threaten the population of *B. robustus* that inhabits the abandoned gravel road at Patersons Terrace. The gravel no longer undergoes regular disturbance because vehicles are now prohibited from driving on the road, and since the management of the land has been transferred to the New Zealand Department of Conservation, grazing of sheep (*Ovis aries*) has been discontinued and lagomorphs have been removed, hence reducing grazing pressure on the road and roadside vegetation. As such, future preservation of *B. robustus* at this site, and other habitats throughout the Mackenzie Basin likely requires the implementation of an artificial disturbance regime and/or weed control to maintain open bare gravel habitat.

Replicating disturbance regimes has been shown to be effective mitigation (Cornelisse et al. 2013) for anthropogenic changes to disturbance regimes that threatens the persistence of other bare ground specialist insects (Stelter et al. 1997, Tockner et al. 2006). For example, the blue-winged grasshopper (*Oedipoda caerulescens*) is an early coloniser of dry sandy grasslands in Germany and prefers habitat with 30 % to 50 % bare ground (Warren and Büttner 2008). Recent changes to disturbance regimes have reduced the area of bare ground in these habitats, however military training activities within some parts of the grasslands maintain essential rates of disturbance and create important habitat refuges for *O. caerulescens* (Warren and Büttner 2008). A change in natural disturbance regime since European settlement of coastal prairies in Santa Cruz Country, California, has also threatened bare ground habitat for the endangered Ohlone tiger beetle (*Cicindela ohlone*) (Cornelisse et al. 2013). Recommended conservation management for this beetle includes regular

ground scraping to create bare areas of new habitat for colonisation by the beetle (Cornelisse et al. 2013). These examples indicate that replicating natural, regular disturbance events could be a vital mitigation action that is required in habitats where natural disturbance processes have been disrupted by human development.

The invasion of open habitats by exotic weedy species is a threat to insects across multiple taxa. In New Zealand, beetles of the *Prodontia* genus primarily inhabit grasslands (Barratt 2007). However, over the past century grasslands have been invaded by exotic woody weeds such as wilding pines (mostly conifer species in the genus *Pinus*), gorse (*Ulex europaeus*) and thyme (*Thymus vulgaris*), as well as herbaceous mat forming weeds such as hawkweed (*Hieracium* spp. and *Pilosella* spp.). Although some *Prodontia* spp. can cope with low density thyme invasion (Emerson 1994), loss of grassland habitat due to the invasion of exotic species threatens the persistence of several other species from this genus (Barratt 2007). In the Mackenzie Basin, where native grasslands have become increasingly invaded by wilding pines, capture rates of insects from the orders Orthoptera, Hymenoptera and Hemiptera were found to be lower when conifer densities were > 800 trees per ha, or ~50 % canopy cover, and Coleoptera diversity was found to have declined by 35 % (Pawson et al. 2010). The loss of native grasslands in the Mackenzie Basin also threatens several native moth species (Patrick 2004). Similarly, in the Czech Republic the invasion of alpine tundra by dwarf pine (*Pinus mugo*), in part due to global warming, has reduced Coleoptera abundance and functional richness (Kašák et al. 2015). On the Crau plain in France, the natural open 'coussou' habitat is becoming increasingly fragmented by intensive agricultural development, and the resulting change in vegetative cover threatens the grasshopper *Prionotropis hystrix rhodanica* which is endemic to the plain (Foucart and Lecoq 1998). These examples contribute evidence that the invasion of low productivity, open habitats by exotic weedy species is a key concern for conservation management of insects across multiple taxa at a global scale.

7.3. Threats

Ensuring that threats to population persistence are not present within the receiving habitat is essential for maximising population growth and achieving conservation translocation success (IUCN 2013). Chapter 4 presented evidence that introduced mammalian predators are likely to pose a substantial threat to *B. robustus,* leading to the recommendation that high intensity mammalian predator control that targets the entire suite of mammalian predators should be implemented in receiving habitats to maximise *B. robustus* population growth and translocation success.

For many New Zealand species threatened by introduced mammals, including the Mahoenui giant wētā, *Deinacrida mahoenui* (Watts and Thornburrow 2009), the absence of mammalian predators at a release site is essential for translocation success. Off-shore mammal-free islands are considered to be the most beneficial and cost-effective receiving habitats for a conservation translocation (Scofield et al. 2011) because of their isolation from predatory mammal populations. However, for some threatened insect species, off-shore islands may not be suitable because they lack the necessary habitat types (e.g. open gravel habitats for *B. robustus*). Predator exclusion fences (Clapperton and Day 2001) that circumference an area of suitable habitat on the mainland are an alternative option, and have been effective at reducing predator pressure during translocations of other vulnerable New Zealand insects (Sherley 1994, Watts and Thornburrow 2009) and invertebrates (Walker 2003). This approach is currently being evaluated for *B. robustus* (L. McIver, T. Murray, unpub. data.) at a 200 m stretch of the gravel road habitat at Patersons Terrace that was fenced at the end of 2018 (Te Manahuna Aoraki DOC, pers. comms.). However, fencing other populations of *B. robustus* that inhabit riverbed is unsuitable because the dynamic nature of the habitat will damage the fence and create opportunities for mammalian predators to pass. A better approach might be to fence an entire catchment, or to implement high intensity trapping such as that implemented in the Upper Ōhau River (Woolmore et al. 2010) that benefits *Sigaus minutus* grasshoppers (Chapter 4). Benefits of expansive mammalian predator control is also likely to extend to threatened insects from multiple taxa beyond the target species. For example, a proportion of the > 900 species of mostly native invertebrates that were found to inhabit a single vegetation community within the Tasman Riverbed (Murray and Anderson 2019) would benefit from extensive predator control implemented across the entire catchment. The importance of eliminating exotic mammalian predators from expansive areas of New Zealand, for example, the entire Mackenzie Basin which is the goal of the Te Manahuna Aoraki project (Te Manahuna Aoraki DOC, pers. comms), is highlighted by the fact that introduced species are one of the four main drivers of insect decline globally (Sánchez-Bayo and Wyckhuys 2019).

Although high intensity mammalian predator control is expected to benefit insect populations and improve translocation success, it is possible that predation pressure might be inflated under these conditions as a result of meso-predator release. As discussed in Chapter 4, lizards, birds and certain invertebrates are also predators of *B. robustus* as well as prey of mammals, and their populations are likely to benefit from mammal suppression or eradication. Native meso-predators are expected to exert less predation pressure on native insects than introduced mammals because many native insects have defence mechanisms such as visual crypsis that limit detection (Gibbs 1998, Lester et al. 2014) and therefore mortality. However, the evidence to support this expectation is mixed. Following the eradication of introduced mammals (except mice) from Maungatautari, a fenced wildlife sanctuary,

populations of wētā showed dramatic increases in relative abundance (Watts et al. 2011). However, within Zealandia, another fenced wildlife sanctuary where all mammals except mice had been eradicated, and Otari-Wilton's Bush, an unfenced sanctuary with sustained mammal control, beetle abundance showed trends of decline (Watts et al. 2014). One explanation was that native insectivorous birds that thrived within the reserves following the suppression of mammals increased predation pressure on insects. Furthermore, invertebrate diversity did not differ inside and outside of Zealandia after 17 years of mammal eradication within the sanctuary (Parra 2018). In part these mixed results might be a consequence of body size, because larger insect species, like wētā (and *B. robustus*), are more strongly targeted by mammalian predators than smaller insect species (St Clair 2011), and their release from mammalian predation pressure could result in a more noticeable benefit. However, other factors including the loss of genetic diversity in extremely threatened species that persist in small populations (Lacy 1997), will also influence species recovery dynamics following the reduction in predation pressure.

The New Zealand government's ambitious goal, '*Predator Free 2050*' (Bell 2016), announced in 2016 has some concerning consequences for threatened insects when considering a potential meso-predator release. The programme's goal is to eradicate mustelids (stoats, *Muestela erminea*; ferrets, *Mustela putorius furo*; weasels, *Mustela nivalis*), rats (ship rat, *Rattus rattus*; Norway rat, *Rattus norvegicus*; kiore, *Rattus exulans*) and possums (*Trichosurus vulpecula*) from the mainland by 2050. However, cats (*Felis catus*), mice (*Mus musculus*) and hedgehogs (*Erinaceus europaeus*) are excluded from the objectives. This is concerning because cats (Pierce 1987, White 1994, Murphy et al. 2004, O'Donnell et al. 2017) and mice (Wilson and Lee 2010) consume insect prey throughout New Zealand, and hedgehogs are primarily insectivores (Jones et al. 2005, Jones and Norbury 2011). For threatened insect species, inflated pressures from a potentially nation-wide meso-predator release (of both mice and native predators), in combination with continued pressure from non-targeted mammals including cats, mice and hedgehogs could lead to steeper decline trajectories than already observed. Targeting the full suite of introduced mammalian predators is likely to be the most beneficial approach for conserving threatened insects in New Zealand, because although it may increase pressure from native and introduced birds, and lizards, it would remove additional pressure from those predators for which native insects have no defences. The result being that predation pressure would more closely reflect natural levels observed prior to the introduction of introduced mammals.

7.4. Monitoring

Conducting post-release monitoring as part of a conservation translocation procedure is vital for determining whether the event has been successful, or for identifying causes of failure and informing future modifications to translocation design (Seddon et al. 2007). Monitoring of source populations is also important for measuring any detriment that removing individuals for a translocation might have had. Beyond translocations, general monitoring of threatened species is important for measuring the benefit that other conservation actions have provided (Lyons et al. 2008), or for triggering the implementation of conservation action once population size or trend becomes undesirable (Block et al. 2001, Cook et al. 2016).

A key finding from Chapter 5 was that detectability of *B. robustus* was strongly correlated with body size; the largest individuals, late instar and adult females, were the most detectable demographics. It was recommended that adult females be used as an index for population size because the large body size results in higher confidence of correct species identification, particularly among inexperienced observers (Fraser 1999), and because adult females are also a biologically informative demographic in that they directly contribute to the next generation. This recommendation applies to monitoring of other species of cryptic grasshoppers both in New Zealand and internationally, because female grasshoppers are almost always larger than male grasshoppers. In New Zealand, evidence suggests peak adult female abundance of two other At Risk grasshoppers, *S. minutus* (Jamieson 1996, Jamieson 1998, Schlump 2018) and *Sigaus childi* (Jamieson 1999), occurs in late spring or early summer, whereas for other widespread and non-threatened species such as *Sigaus campestris* (Northcroft 1967) and *Brachaspis collinus* (Batcheler 1967), the seasonal peak in adult female abundance is more ambiguous. A rapid assessment of population demographics within a season, such as that presented in Chapter 5, is recommended for establishing optimal timing for monitoring to occur.

A second key finding was that long-term population trends generated from multiple years of monitoring are likely to be more informative than single year-to-year comparisons for insects including *B. robustus* (Chapters 5 and 6). One reason why a year-to-year comparison is not recommended for *B. robustus* is that it takes 2 years from when an egg is laid to when the resulting grasshopper reaches reproductive maturity (Chapter 2). Therefore, comparisons between adult populations in consecutive years are not very informative because they are mostly reproductively separated. For other species of New Zealand alpine grasshoppers, the time to reach adulthood under natural conditions is also likely to be > 2 years from the time an egg is laid because of slow growth rates (Batcheler 1967, Hudson 1970) and obligatory egg diapause (Mason 1971). For these grasshoppers, and other insects that show

similar life history traits to *B. robustus,* long-term monitoring of populations over > 6 years (approximately 3 generations) will be necessary to interpret population trends with confidence. For annual grasshoppers such as *Phaulicridium marginale* (Northcroft 1967), single year-to-year comparisons may be more useful. However, counts are still vulnerable to potentially large annual fluctuations driven by favourable or unfavourable conditions (Dempster 1963). Comparing population size in a particularly favourable year to population size in an average year provides a measurement of population fluctuation, but it does not represent long-term trend and therefore provides little valuable information for conservation decision making. In general, interpreting population trends using data that is 'long-term' relative to life expectancy and time to reproductive maturity is recommended.

A third key finding was that population monitoring surveys required replication in both space and time. Adequately replicating monitoring in space is important for insects (including *B. robustus*; Fraser 1999) that are patchily or unevenly distributed across space, particularly if they are clumped around resources including host plants or vegetative structures (e.g. butterflies; Harker and Shreeve 2008). Even when sampling is adequately replicated in space, it is also important that sampling is adequately replicated in time to overcome detection errors, such as false absences where an individual is present but not sighted by the observer (Dennis et al. 1999, Dennis et al. 2010). These errors can arise from crypsis (e.g. coloration that visually mimics the habitat or another species) and elusive behaviour (e.g. refuge seeking, or freeze responses), species phenology (e.g. timing of egg or larvae phases, or peak adult abundance; Harker and Shreeve 2008) and environmental influences over short time frames (e.g. lack of activity during cold weather, or differences in activity related to time of day; Harker and Shreeve 2008). Because accurately estimating population size is vital for conservation decision making, yet detection of threatened insects is almost certainly prone to errors, adequately replicating surveys in space and time is a fundamental component of threatened insect monitoring design.

7.5. Future research directions

7.5.1. Maximising genetic diversity of founder populations

One consideration that has not be explored in this thesis is the genetic consequence of establishing small founder populations. Founder populations are formed from a subset of one or more source populations, meaning they undergo a human-induced demographic bottleneck at the time of translocation that may result in a founder effect (i.e. founding populations that contain a sub-sample of the genetic diversity of source populations; Frankham et al. 2010). These small founder populations

are vulnerable to further genetic diversity loss due to inbreeding and genetic drift (Frankham et al. 2010, Jamieson 2010). These effects can reduce individual and population fitness in the short-term and limit the potential for founding populations to adapt to future environmental change in the long-term (Keller and Waller 2002, Witzenberger and Hochkirch 2008). To mitigate this, early translocation literature suggested using large numbers of individuals to found new populations to improve both short-term and long-term success (Griffith et al. 1989). One issue with this approach is that threatened species, by definition, have few individuals available for translocation and moving a large proportion of them could be detrimental to source population(s), especially if the risk of translocation failure is high (Ottewell et al. 2014).

To maximise success and conservation benefit, conservation translocations should strategically balance the number of founding individuals to maintain adequate levels of genetic diversity in founding populations with minimal detriment to the integrity of wild source populations. Tools that help inform conservation managers making this decision include simulation software such as *AlleleRetain* (Weiser et al. 2012). *AlleleRetain* simulates demography and inbreeding to predict the persistence of rare alleles within small populations over time (Weiser et al. 2012). The model requires input about the source population, the individuals released into the founding population, the characteristics of the founding population, and the life history of the species (Weiser et al. 2012). The program can be used for determining the optimal number of founders, and the frequency at which translocated populations should be augmented to minimise the loss of genetic diversity over time (Weiser et al. 2012).

Using an *AlleleRetain* simulation is recommended to evaluate the optimal number of grasshoppers to release in any future translocations and determine whether the optimal release strategy requires augmentation. Many of the life history traits required for running the simulation for *B. robustus* were explored in Chapter 2. Although the characteristics of the founding population, including demographics and population growth, have not been presented here, they can potentially be determined from two observations. Firstly, population size and demographic data collected following the release of grasshoppers in the initial translocation in 2015 can be used to estimate the characteristics of founding populations released into artificial, or human created habitats. Alternatively, continued monitoring of the Snowy River population to assess population recovery subsequent to the flood event in February 2018 (Appendix B, see also section 7.5.3), could provide population growth parameters for grasshoppers released into more natural habitats (i.e. braided rivers in the Mackenzie Basin). Further, simulations could potentially be used to compare allele retention under conditions associated with natural and artificial release habitats. Although *AlleleRetain* is a useful tool for translocation decision making, other factors, including the

consequences of extirpating the source populations, still require consideration to achieve maximum conservation benefits.

7.5.2. Disease and parasite identification

Another important consideration in a translocation procedure is the role of infectious diseases and parasites (IUCN 2013). Infectious diseases and parasites (including viral, bacterial, fungal, protozoan, and metazoan) are common among insects, and may cause individuals to die, have supressed reproductive capacity and/or an increased susceptibility to further disease (Cunningham 1996). Translocated populations are vulnerable to disease outbreaks because they are small with limited diversity, and the translocation procedure itself can stress individuals and increase their susceptibility to disease (Teixeira et al. 2007, Sainsbury and Vaughan-Higgins 2012). If a translocation introduces a disease to a naïve population (e.g. during a reinforcement), or individuals for a founding population are sourced from multiple different populations, including some populations naïve to disease, then negative consequences of disease may outweigh the conservation benefit intended (Cunningham 1996). Minimising disease and parasite transfer during a translocation is often achieved by maximising food and handing hygiene protocols (Cunningham 1996), by screening individuals for disease before release (Alberts et al. 1998), or in cases where vaccinations and medications have been developed, by treating animals before they are released (Viggers et al. 1993, Northover et al. 2018).

Managing parasites during a translocation produces somewhat of a conundrum because although most regulate host population size, which can be detrimental for conservation goals, the parasites also form an important part of biodiversity and may be worthy of conservation themselves. Host-specific parasites of threatened species, such as the ectoparasite *Felicola (Lorisicola) isodoroi* that lives on the endangered Iberian lynx, *Lynx pardinus* (Pérez et al. 2013), are often rarer than their host (Jørgensen 2015, Northover et al. 2018). Preserving rather than eliminating host-specific parasites during a translocation procedure will be important for conserving biodiversity beyond the target species (Moir et al. 2012).

Globally, diseases and parasites that infect grasshoppers are diverse and include protozoa, viruses, fungi, bacteria, rickettsiae and nematodes (Chapman and Joern 1990). Mermithids, mites, cestodes gregarines and egg parasites have been identified as affecting other species of *Brachaspis* grasshoppers in New Zealand (Mason 1971). Some of these parasites (e.g. mermithids and tracheal mites) can potentially reduce ovary development in female grasshoppers (Mason 1971) and have the potential to reduce reproductive output and hinder population establishment during a translocation. Some, including *Beauveria* that was identified as a cause of death for several *B. robustus* grasshoppers

kept in captivity (Chapter 2), are so detrimental to insect populations that they are used as biocontrol agents for pest species (Lomer et al. 2001).

Apart from external mites and *Beauveria,* currently no other diseases or pathogens have been identified for *B. robustus*. Their identification is the first step in developing disease management strategies that maximise conservation outcomes of *B. robustus* and any host-specific parasites during a translocation. Knowing which infectious diseases are common, how they spread, and where they currently persist will inform whether translocations between populations to supplement genetic diversity or population size will be beneficial, or if it will introduce potentially harmful new diseases or parasites to a naïve population (Hartley and Sainsbury 2017).

Suggested research questions include:

1) **Did any diseases or parasites cause death or reduced reproductive output of grasshoppers held in captivity during this study, and if so, what was the frequency of infection?**
   If any diseases or parasites did cause death or reduced reproductive output of grasshoppers in captivity, then it will be important that those diseases and/or parasites are not present in founder populations to maximise population growth upon release and therefore translocation success. Additionally, protocols will be required for managing the spread of infection in a captive environment.
2) **Are there *B. robustus* diseases and parasites that can be identified through faecal matter, regurgitant screening or other non-invasive screening?**
   If diseases and parasites can be non-invasively identified, then disease screening could be incorporated as a pre-translocation protocol to assess the health of individuals.
3) **Do diseases and parasites occur at different frequencies in different populations of *B. robustus* throughout the Mackenzie Basin?**
   If diseases and parasites do occur at different frequencies in different populations of *B. robustus*, then a translocation that sources individuals from multiple populations might have an increased risk of failure if individuals from some populations are naïve to diseases or parasites carried by individuals from another.
4) **Does *B. robustus* support any host-specific parasites?**
   If *B. robustus* does support host specific parasites, then conservation of those parasites will be important for preserving biological diversity.

7.5.3. Impacts of stochastic weather events

In February 2018, ex-tropical cyclone Gita passed over New Zealand and delivered torrential rains across the country. The rainfall resulted in a severe flooding event in the Snowy River that removed approximately three-quarters of the existing *B. robustus* population (Appendix B). Very little is understood about the importance of flooding events for population dynamics, or how *B. robustus* populations recover.

The disturbance caused by severe flooding events is an inherent part of braided rivers and is often important for maintaining habitat patches and population dynamics for braided river species. Severe flooding events are essential for maintaining meta-population dynamics for *Bryodema tuberculata*, a braided river grasshopper from the Northern Alps of Europe (Stelter et al. 1997). Although the floods remove some populations, they also create new bare gravel habitats for colonisation. An absence of flooding events is detrimental for *B. tuberculata* because grasshopper populations are eliminated by habitat loss caused by vegetative succession (Stelter et al. 1997). Flooding events were also found to be important for another critically endangered river grasshopper *Chorthippus pullus* that lives on the dammed Isar River in Germany, because the floods remove vegetation and open up migration corridors that facilitate geneflow between populations (Maag et al. 2013). Historically, major flooding events in the braided rivers that *B. robustus* inhabits were likely to have driven meta-population dynamics similar to those recorded for *B. tuberculate* (Stelter et al. 1997) and *C. pullus* (Maag et al. 2013). However, given that *B. robustus* now occurs in small populations at much lower densities, and populations are no longer well connected, severe events could now be detrimental if they remove substantial proportions of the population. This is concerning because the number of annual large flooding events is predicted to double in major catchments in the Mackenzie Basin over the next 70 years (Caruso et al. 2017).

Monitoring the aftermath and recovery of the *B. robustus* population following the flooding event could provide some useful insights for conservation management. Measuring population growth rate and recolonisation could be useful for determining the importance of maintaining populations across multiple riverbeds for resilience against flooding events. Furthermore, they could provide useful data required for predicting the outcome of a translocation, or could be used for estimating demographic parameters of a translocated population in the *AlleleRetain* model discussed in section 7.5.1.

Determining the life stage that is important for colonising new habitat patches for *B. robustus* could also inform optimal life stages for translocation. Mostly small juveniles were lost during the flood at Snowy River (Appendix B), however it is not known whether they died, or whether a proportion of them were transported downstream. Although downstream drift of *C. pullus*

grasshoppers (carried by water currents) did not contribute to downstream colonisation, it was speculated that eggs being washed downstream may survive and that the subsequent nymphs might colonise new habitat (Maag et al. 2013). It is not known whether *B. robustus* eggs were transported downstream during flooding events or whether they remained viable, but observations that the pod structure disintegrates when damp (Chapter 2) suggests that the eggs become vulnerable to damage during flooding events that restructure the gravel.

Some suggested research questions include:

1. **Do *B. robustus* eggs remain viable after time spent submerged in water?**
2. **Does downstream drift facilitate colonisation of new habitat patches?**
3. **Which demographic colonises open gravel patches following a major flooding event?**
4. **How long did the population at Snowy River take to recover from the flooding event?**

7.6. Concluding remarks

This study has contributed knowledge that has significantly advanced the understanding of a Nationally Endangered insect and provided evidence-based management recommendations to improve translocation success of threatened insects. It provides an example of how investing in understanding species biology, life history traits and habitat use can inform optimal release strategies to establish resilient populations during a translocation, and how removal sampling can be used as a basis for the rapid development of a population monitoring protocol. By determining that introduced mammalian predators threaten dryland grasshoppers, this research has contributed to the documentation of impacts that predatory mammals have had on native and endemic fauna since their introduction to New Zealand. It also provided evidence that high intensity mammalian predator control is important for conservation management of large threatened insects. This study also identified several protocols for improving the accuracy of estimating relative population size during population density monitoring that can be adapted and applied during monitoring of other Orthoptera and similarly cryptic insect species in New Zealand and overseas.

Turning the tide on the imminent mass extinction of insects (Thomas et al. 2004, Régnier et al. 2015, Sánchez-Bayo and Wyckhuys 2019) likely requires action on landscape scales that targets expansive areas, providing benefits to multiple species simultaneously and preserving species' interactions (Samways 2018). However, such extensive conservation management can take a long time and be expensive to implement, and in some situations the benefit may not occur before the

functional or absolute extinction of an individual species takes place. In these situations, a conservation translocation may be required to secure the species for the foreseeable future. In recognition that translocations are disproportionately applied for vertebrate conservation (Fischer and Lindenmayer 2000, Bajomi et al. 2010), the research presented in this thesis advocates for translocation as a conservation strategy for insects, and contributes essential knowledge for developing translocation into a successful and valuable tool for preventing further insect extinctions.

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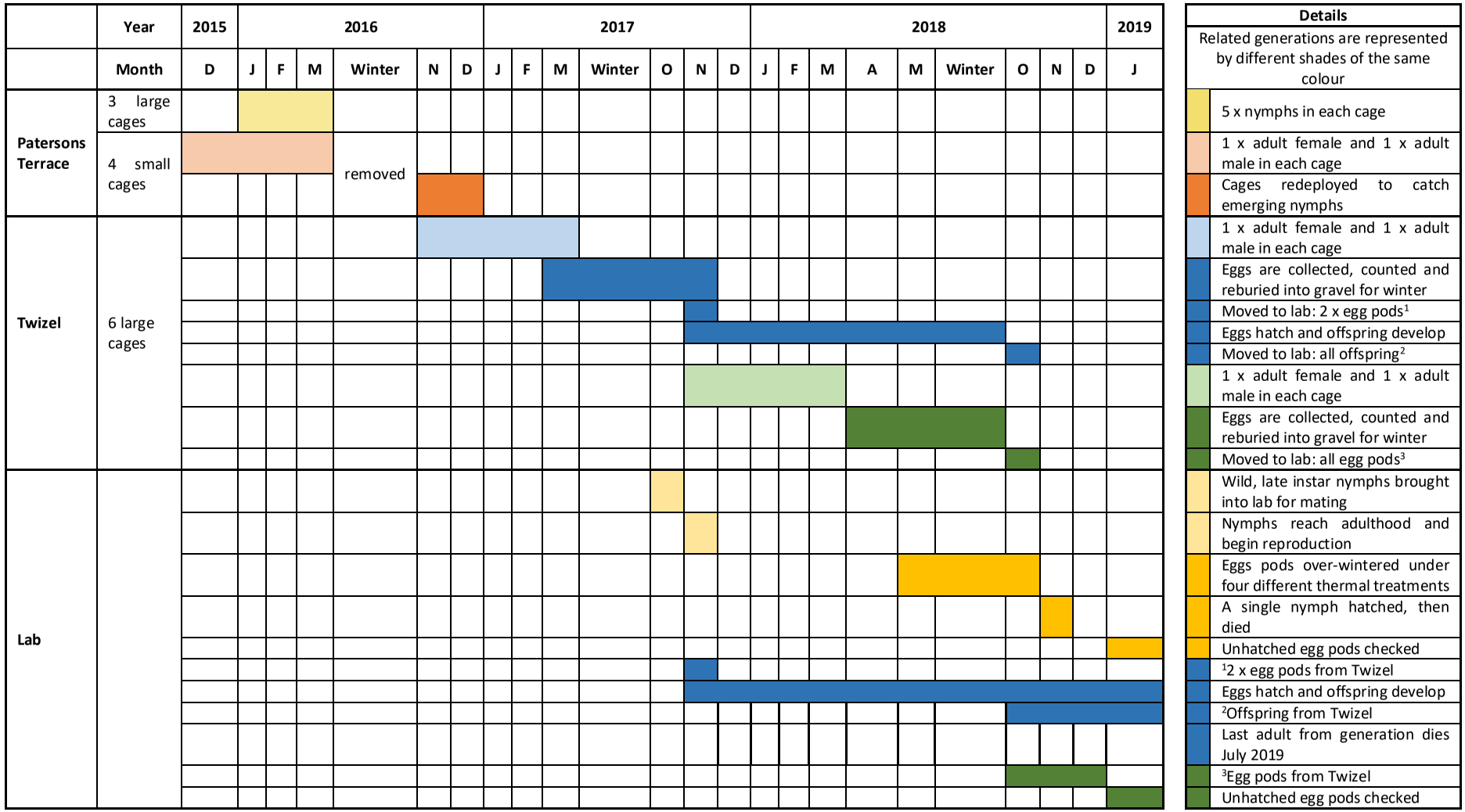
Appendix A

Timeline of the *Brachaspis robustus* life history experimental procedure between December 2015 and January 2019



Appendix B

Changes to a terrestrial braided river insect population after a major summer flooding event

Preface

In the final season of my field work at Snowy River (2017-18), a significant flooding event occurred in February when ex-tropical cyclone Gita brought torrential rainfall. Because regular monitoring was already in place, I was able to record changes in *B. robustus* population demographics before and after the flooding event and compare it to the same period in the previous year. I found the flood caused substantial changes to the population size and composition and have presented those findings in this appendix. I plan to expand on the current work that is presented here by collaborating with Liam McIver, a current MSc student at the University of Canterbury. His work has recorded the recovery of the population one year after the flooding event. I also plan to collaborate with climate modellers at the New Zealand Department of Conservation so that I can discuss conservation management of *B. robustus* and other terrestrial braided river invertebrates in the Mackenzie Basin, in the context of predicted future trends for extreme rainfall events in the area.

Changes to a terrestrial braided river insect population after a major summer flooding event

Introduction

Characterised by highly variable flows and multiple channels that weave across wide gravel floodplains, braided rivers in the Mackenzie Basin of New Zealand provide a unique and dynamic habitat for many threatened, endemic species (Caruso 2006, Caruso et al. 2013). Events of high flow or flood are the driver of the constantly changing nature of these ecosystems. They are also responsible for displacing or completely removing populations of plants and animals that persist in the diversity of habitats that braided riverbeds provide (Gray et al. 2006, Caruso et al. 2013), as well as creating new open habitats for colonisation (Stelter et al. 1997). Understanding how flooding events impact populations of braided river species and the time required for population recovery has valuable implications for the management of threatened species.

Inhabiting the braided rivers of the Mackenzie Basin is the Nationally Endangered endemic robust grasshopper, *Brachaspis robustus* (Stringer and Hitchmough 2012, Trewick et al. 2012). Unlike most grasshopper species, it prefers the terrestrial rocky substrate of fluvial outwash found in braided riverbeds. It is a large, flightless, sexually dimorphic insect (males up to 17 mm and females up to 38 mm in body length) with a two-year life cycle that results in overlap between life stages of different generations. Eggs and juveniles at different stages of development can be present at any point in the year (White 1994). In late summer (February – March), populations of *B. robustus* consist mostly of mid- to late-instar juveniles (Chapters 5 and 6) and recently laid eggs of the preceding generation buried in the substrate (Chapter 2). Here we report on the impacts of a major summertime flooding event caused by ex-tropical cyclone Gita on a population of *B. robustus* that was monitored for four weeks before and after the flood event.

Methods

On the 20th and 21st of February 2018, ex-tropical cyclone Gita crossed New Zealand bringing with it extreme rainfall. The Snowy River in the Mackenzie Basin, an alluvial fan with braided river characteristics, went into unseasonal flood (Figure B.1). This was the highest rainfall event to occur in February in the past 20 years (Figure B.2), at any time of the year since 2009 (Figure B.3), and in the summer months (December to February) since 2004 (Figure B.3). A population of *B. robustus* inhabit

a usually dry reach of the Snowy River and were monitored between 22nd November 2016 and 30th March 2017, and 10th November 2017 and 20th March 2018, including immediately before and after the flooding event caused by ex-cyclone Gita.

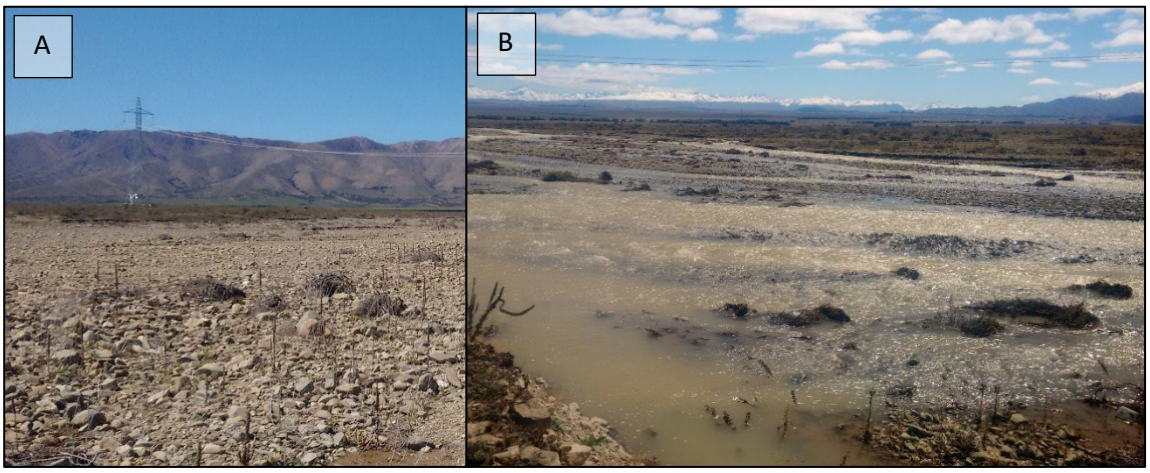


Figure B.1. (A) The dry reach of the Snowy River where a population of *B. robustus* was monitored, pictured facing upstream/east. (B) The same reach of the Snowy River on 22nd February 2018, pictured facing downstream/northwest, one day after ex-tropical cyclone Gita brought torrential rainfall to the area.

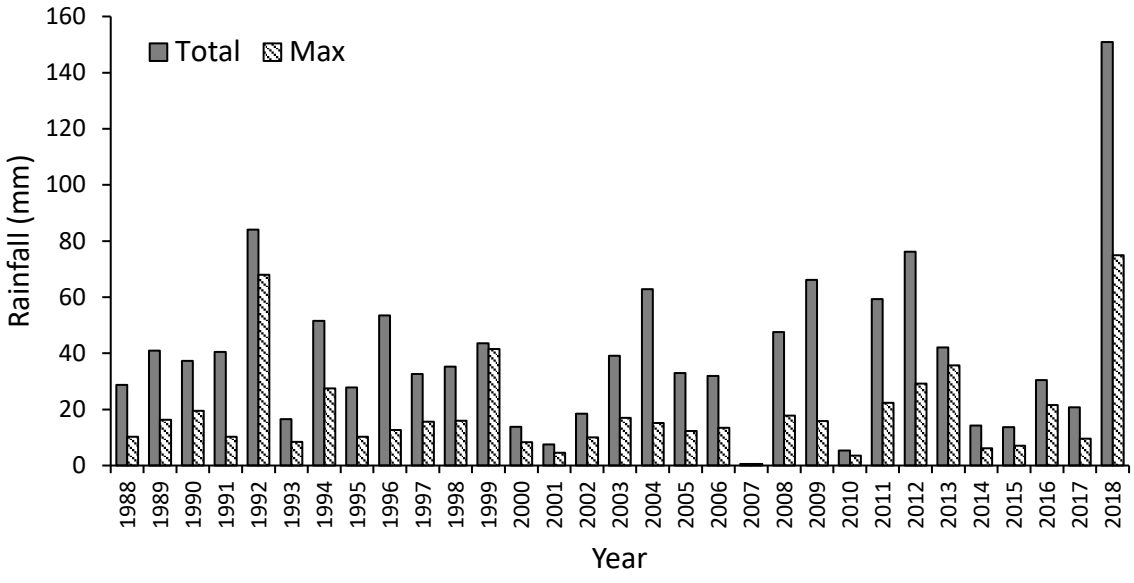


Figure B.2. The total monthly (mm per month), and maximum daily (mm per 24-hours), rainfall for February between years 1988 and 2018 recorded at Air Safaris Station, Lake Tekapō, ~25 km NNE from the Snowy River. Data sourced from the NIWA National Climate Database.

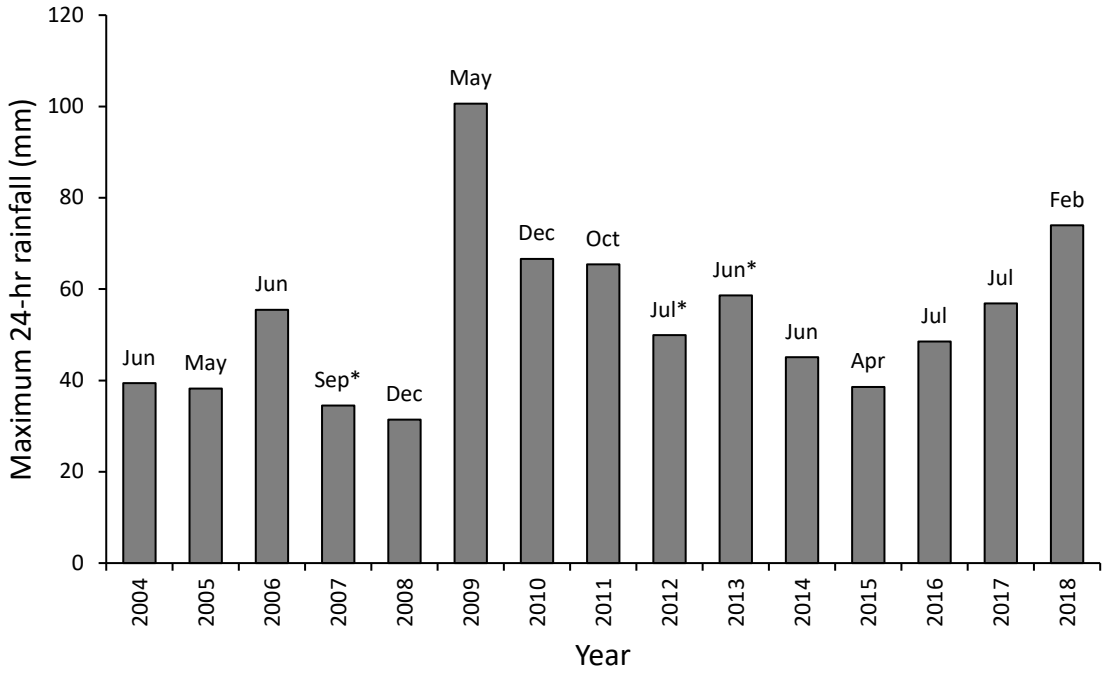


Figure B.3. The maximum daily (mm per 24-hours) rainfall per year since records by NIWA began at Lake Tekapō Ews (~25 km NNE from the Snowy River) in 2004 until 2018. Data labels indicate which month the event was recorded in. * indicates that data from one or more months is missing from the database for that year. Data was sourced from the NIWA National Climate Database.

Three 100 m x 1 m transect lines were set up on the Snowy River in 2016-17, and five in 2017-18, spaced at approximately 200 m intervals along a 1 km reach of the riverbed. The transects were walked by a single observer at a steady pace on days of suitable weather (ground temperature >14 °C and not during high winds or precipitation). Ground temperature in the shade and relative humidity were measured at the start of each monitoring event using a Kestrel 3500 Pocket Weather Metre (GeoSystems New Zealand Ltd). Monitoring of all transects usually took less than one hour. A total of 11 monitoring days were conducted during the 8-week period between 25th January and 2nd March 2017, and 14 days between 23rd January and 20th March 2018. Monitoring did not take place between 22nd – 25th February 2018 because the transects were inundated with flood water. On February 26th normal monitoring resumed. All *B. robustus* sighted on the transect were captured. Their body length (from the top of the head to the tip of the abdomen), femur length and sex were recorded before they were released again, behind the observer, at the exact location of capture. Body length was not recorded between 21 February and 30 March 2017 but was imputed using femur length which has been shown to be correlated to body length for this species (Chapter 5). Transect counts were pooled together for each day to give an average count per 300 m^2 2017 and per 500 m^2 in 2018. Population

size, body and femur length of males and females, ground temperature and relative humidity were compared before and after the flooding event in 2018, and in the same "before" period and "after" period in 2017 using bootstrapping to account for small sample sizes ($n < 30$). All analyses were carried out in *R* (R Development Core Team 2011).

Results

The rainfall brought to Snowy River by ex-cycle Gita caused on average 73 % (± 0.14) of each transect to become inundated with flowing water. The average number of grasshoppers found was significantly lower after the flood (2.9 per day) compared to before (10 per day, p = 0.003). Across the same period in the previous year there was no significant change in the number of grasshoppers present (before = 8.5, after = 11.2, p = 0.22; Figure B.4).

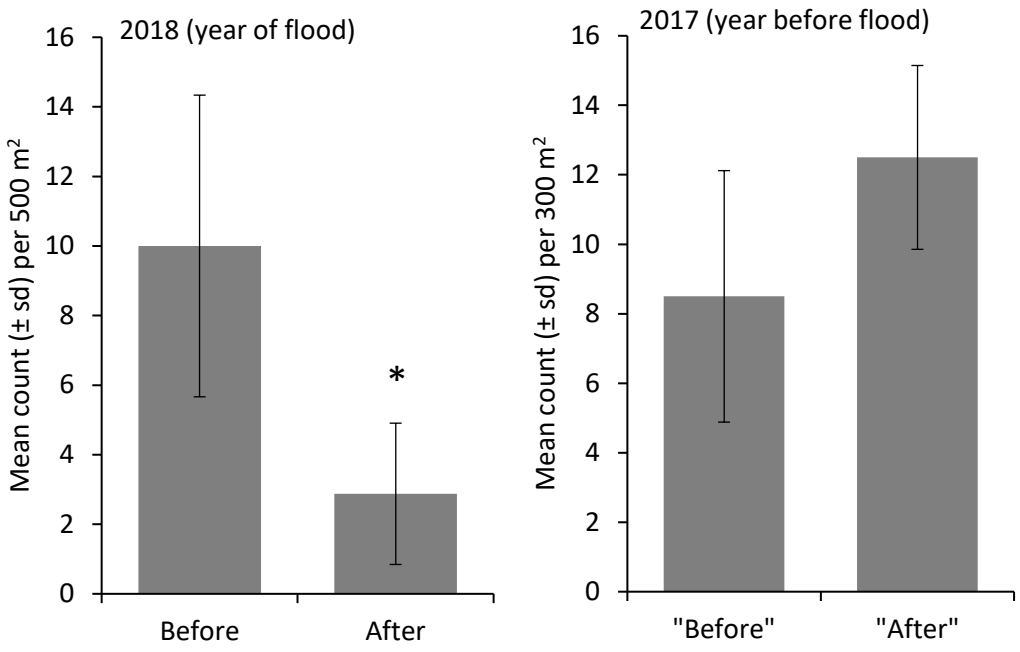


Figure B.4. The mean (± sd) count of *B. robustus* per 500 m^2 in Snowy River in the four weeks before and after the 22nd February when a major flooding event caused by ex-cyclone Gita occurred in 2018 (left). The mean (± sd) count of *B. robustus* per 300 m^2 in the equivalent four-week period before and after in 2017 (right). * denotes $p < 0.05$. Number of sampling days in 2018, before $n = 6$, after $n = 8$; and 2017, "before" $n = 6$, "after" $n = 5$.

In the four weeks prior to the flood in 2018, 47 % of the grasshoppers sighted were females, 37 % were males, and 17 % were too small to determine sex or measure femur length, compared to 91 % females and 9 % males after the flood (Figure B.5). In the equivalent four week "before" period

in 2017, 46 %, 42 % and 12 % of grasshoppers sighted were female, male and unknown respectively, compared to 50 % female, 42 % male, and 8 % unknown in the equivalent “after” period.

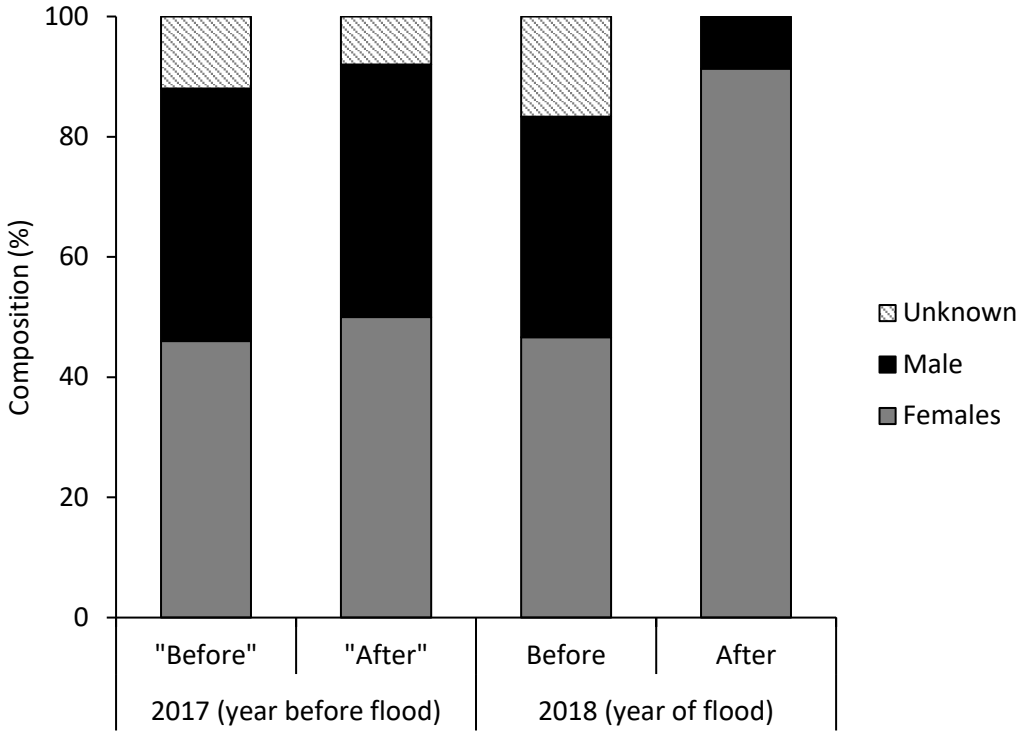


Figure B.5. The sex composition of the *B. robustus* population monitored in the Snowy River in the four weeks before, and the four weeks after the flooding event caused by ex-cyclone Gita in 2018, and the composition over the equivalent periods “before” and “after” in 2017, the year prior to the flood. Unknown = juveniles that were too small for sex to be determined. Total number of individuals in 2018, before *n* = 60, after *n* = 23; and 2017, “before” *n* = 50, “after” *n* = 55.

Mean male body length did not significantly differ before (15 ± 3 mm) and after (15 ± 2 mm, p = 0.71) the flood, but females were significantly larger after (18 ± 4 mm) compared to before (before = 15 ± 3 mm, p = 0.008). Similarly, mean male femur length did not significantly differ before (7 ± 1 mm) and after (8 ± 1 mm; p = 0.66) the flood, but female femur length did (before = 8 ± 2 mm, after = 10 ± 2 mm, p = 0.004). During the previous year, femur length in the equivalent “before” period significantly differed from the “after” period for males (before = 6 ± 1 mm, after = 8 ± 1 mm, p < 0.001), and females (before = 6 ± 1, after = 9 ± 2, p < 0.001; Figure B.6). Ground temperature and relative humidity did not significantly differ during monitoring events before and after the flood (mean temperature; before = 28.7 °C, after = 24.7°C, p = 0.07. Mean relative humidity; before = 32.2 %, after = 40.7 %, p = 0.19).

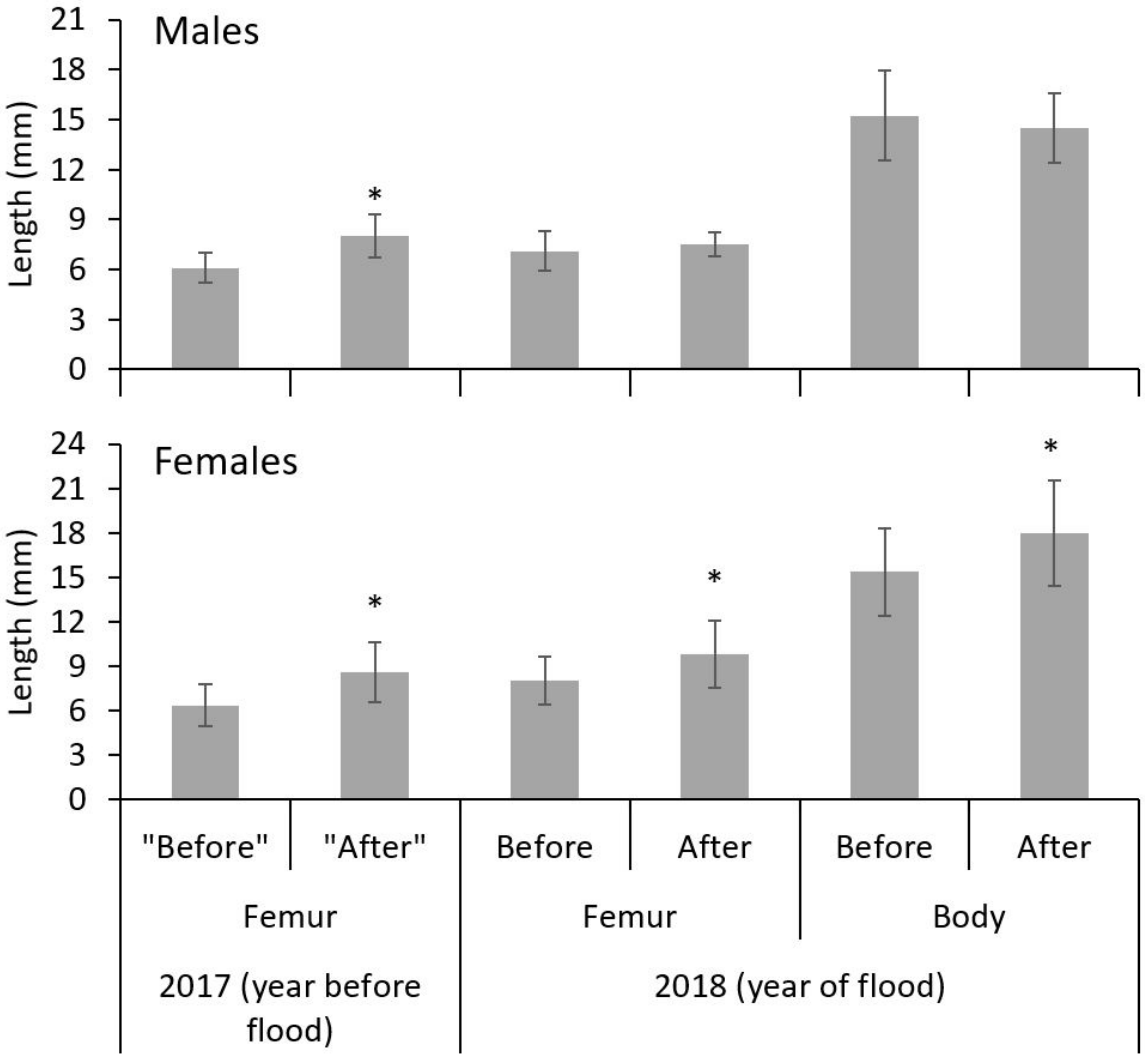


Figure B.6. The mean body length (mm) and femur length (mm) of male (top) and female (bottom) grasshoppers at Snowy River in the four weeks before and four weeks after the flooding event on the 22nd February 2018 caused by ex-cyclone Gita, and in the same period in the year previous. * denotes $p < 0.05$.

Discussion

Approximately three-quarters of the *B. robustus* population in the Snowy River appear to have been displaced or killed during the severe summertime flooding event in February 2018. Such a high loss of individuals following a flood is not uncommon for insect species inhabiting rivers, although most studies focus on the benthic insect community (Scrimgeour et al. 1988, Hendricks et al. 1995). Scrimgeour et al. (1988) found that in New Zealand braided river benthic insect communities can

recover within 132 days of the flooding event. This rapid recovery is likely because many benthic insect species have short life cycles. For example, mayflies (*Deleatidium* spp.) can recover within 132 days (Scrimgeour et al. 1988) but are on average bivoltine (Huryn 1996). For longer lived insects including *B. robustus,* which is semivoltine with a generation time closer to 2- 2.5 years (Chapter 2), the rate of population recovery is likely to be much slower (Beketov et al. 2008).

The unpredictable nature of flooding events in New Zealand's braided rivers is thought to be a driver of the asynchronous life cycle seen in many New Zealand benthic insect invertebrates (Winterbourn et al. 1981) because it provides "insurance" for survival (Danks 1992). Although *B. robustus* has an unsynchronised life cycle (Chapter 2), it is unclear which life stage provides resilience to flooding events. In Germany, the egg stage of another grasshopper restricted to gravel riverbed habitat, *Bryodema tuberculate*, is resistant to flooding provided the substrate is not restructured (Reich 1991). The riverbed substrate at Snowy River underwent high disturbance during the February 2018 flood, so it seems likely the eggs would have been displaced downstream or destroyed. The gravel habitat ends a short distance (< 2 km) downstream from where the population that was monitored occurs and the *B. robustus* population does not extend much further above the monitored site either.

The data presented here indicate that males were more likely to be displaced or killed during the flood than females. In itself, the substantial loss of males is interesting because it is not frequently reported in the literature how flooding events affect different sexes. However, because males are smaller than females, it is unclear whether the key driver of this observation is body size or sex. A major caveat when interpreting the comparison of body sizes and femur lengths before and after the flood is that most of the population were juveniles at the time of this study. Juveniles measured after the flood are older and expected to be larger than those measured before the flood because of their continued development over the eight weeks of monitoring. This is one explanation for the observation that females were significantly larger after the flood. It is also expected that male body size might not differ before and after the flood because they are much smaller than females and a difference would be harder to detect. Comparing trends in body or femur lengths across the same period in the previous year is also problematic because growth rates of grasshopper nymphs are driven primarily by environmental conditions such as temperature (Clissold and Simpson 2015). Juveniles before the flood already had longer femur lengths than juveniles in the equivalent 'before' period in the previous year indicating that they were already somewhat more developed, limiting comparisons in sizes between the two years.

There are limited opportunities to study the recovery of *B. robustus* populations following a rainfall flooding event. Most other populations of *B. robustus* occur in rivers that are flow regulated

by hydro-electric dams, and normal flood regimes from rainfall or spring snow melt have been disrupted. High flows only occur in these rivers when water is spilled over the dams from the hydro lakes. These controlled flooding events might exert different pressures on *B. robustus* populations compared to historical or natural flooding events given they likely differ in magnitude, frequency and timing within the season and considering that different life stages, ages or sexes could be more vulnerable to flooding events than others. Monitoring the recovery of the *B. robustus* population in the Snowy River will provide some important insights into the resilience of this species to mid-summer high disturbance flooding events. The results presented here indicate that conservation of the species will require management of multiple *B. robustus* populations across several rivers to minimise the risk of population decline following any future severe flooding events.

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Appendix C

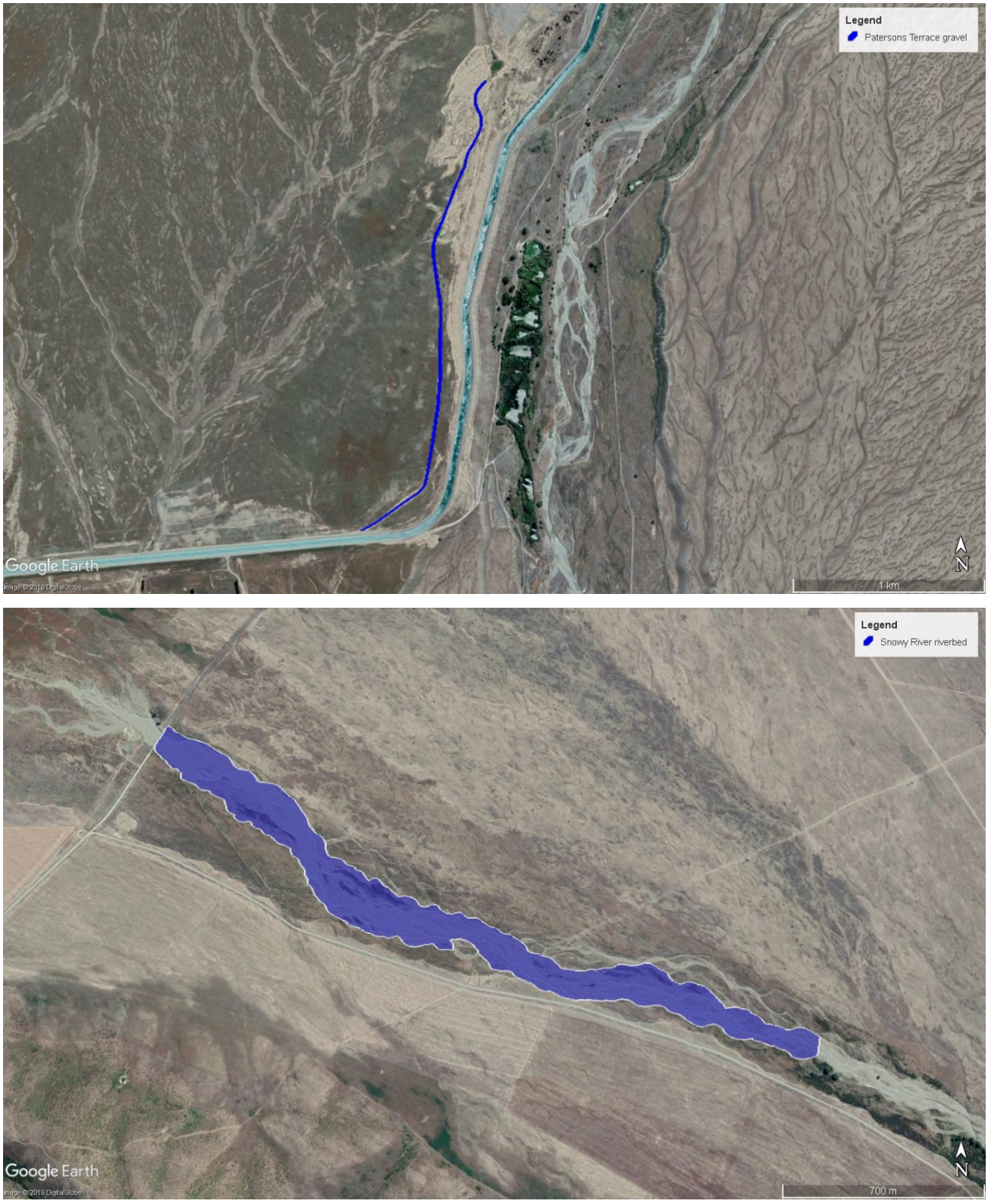


Figure C.1. The area of habitat considered to be occupied (blue shading) when estimating *Brachaspis robustus* population sizes at Patersons Terrace (17,501 m^2, top) and Snowy River (359,996 m^2, bottom).

Appendix D

Table D.1. Post hoc pairwise comparisons of p on a 20 m^2 transect at Patersons Terrace and Snowy River in November, December, January, February, March under no cloud, high cloud, patchy cloud and overcast weather conditions. Common letters denote no significant difference at $p < 0.05$.

	Location	Month	Sky	lsmean	SE	df	asymp.LCL	asymp.UCL	group
22	Snowy River	Dec	overcast	-2.78615	0.33036	Inf	-3.84994	-1.72236	A
30	Snowy River	Nov	overcast	-2.49943	0.341736	Inf	-3.59985	-1.39901	A B
28	Snowy River	Mar	overcast	-2.23393	0.328344	Inf	-3.29122	-1.17663	A B C D
21	Patersons Terrace	Dec	overcast	-2.02159	0.332224	Inf	-3.09138	-0.95179	A B C E F G H
2	Snowy River	Dec	high cloud	-1.98848	0.31988	Inf	-3.01852	-0.95844	A B C D F I J K
26	Snowy River	Jan	overcast	-1.96372	0.328508	Inf	-3.02154	-0.90589	B C D E F G I J K L M N O
32	Snowy River	Dec	patchy cloud	-1.93137	0.329969	Inf	-2.9939	-0.86884	A B C D E I L M
24	Snowy River	Feb	overcast	-1.91929	0.328229	Inf	-2.97622	-0.86237	B C D E F G I J K L M N O
29	Patersons Terrace	Nov	overcast	-1.73487	0.343666	Inf	-2.8415	-0.62823	A B C D E F G H I J L N P Q R
10	Snowy River	Nov	high cloud	-1.70176	0.336223	Inf	-2.78443	-0.61909	A B C D E F G H I J K L M N O P S T U
40	Snowy River	Nov	patchy cloud	-1.64465	0.338519	Inf	-2.73471	-0.55458	B C D E F G H I J K L M N O P Q S V W X Y ZA
12	Snowy River	Dec	no cloud	-1.54664	0.300327	Inf	-2.51372	-0.57956	B C D E F G H I J K L M N O P S T U W Y ZB ZC ZD ZE ZF ZG ZH ZI ZJ
27	Patersons Terrace	Mar	overcast	-1.46936	0.330599	Inf	-2.53392	-0.40481	B C D E F G H I J K L M N O P Q R S V ZB ZK

8	Snowy River	Mar	high cloud	-1.43626	0.313589	Inf	-2.44604	-0.42647	C D E F G H I J K L M N O P Q R S T U V W X ZB ZC ZD ZF ZG ZI ZK ZL ZM ZN ZO ZP
38	Snowy River	Mar	patchy cloud	-1.37915	0.32248	Inf	-2.41756	-0.34073	C D E F G H I J K L M N O P Q R S T V W X Y ZA ZB ZC ZD ZE ZF ZH ZK ZL ZM ZN ZQ ZR
20	Snowy River	Nov	no cloud	-1.25992	0.31026	Inf	-2.25898	-0.26085	C D E F G H I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT
1	Patersons Terrace	Dec	high cloud	-1.22392	0.330328	Inf	-2.2876	-0.16023	B C D E F G H I J K L M N O P Q R S T U V ZB ZF ZI ZK ZM ZO
25	Patersons Terrace	Jan	overcast	-1.19915	0.329534	Inf	-2.26028	-0.13802	D I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS
31	Patersons Terrace	Dec	patchy cloud	-1.1668	0.340701	Inf	-2.26389	-0.06972	B C D E F G H I J K L M N O P Q R S V W X Y ZA ZB ZC ZE ZK ZL ZQ
6	Snowy River	Jan	high cloud	-1.16605	0.312066	Inf	-2.17093	-0.16117	E G H L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT

23	Patersons Terrace	Feb	overcast	-1.15473	0.330331	Inf	-2.21842	-0.09103	I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS
4	Snowy River	Feb	high cloud	-1.12162	0.311263	Inf	-2.12392	-0.11933	E G H L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT ZU
36	Snowy River	Jan	patchy cloud	-1.10893	0.317607	Inf	-2.13166	-0.08621	F G H J K N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT ZV
34	Snowy River	Feb	patchy cloud	-1.06451	0.317816	Inf	-2.0879	-0.04111	F G H J K N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT ZU
18	Snowy River	Mar	no cloud	-0.99442	0.287423	Inf	-1.91995	-0.06889	H P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT ZU
9	Patersons Terrace	Nov	high cloud	-0.9372	0.346305	Inf	-2.05233	0.177936	C D E F G H I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT
39	Patersons Terrace	Nov	patchy cloud	-0.88008	0.349114	Inf	-2.00426	0.244093	DJ K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ

									ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT ZU
	11	Patersons Terrace	Dec	no cloud	-0.78208	0.307499	Inf	-1.77225	0.208098	N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT
	16	Snowy River	Jan	no cloud	-0.72421	0.288305	Inf	-1.65257	0.20416	QR V X ZA ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT ZU ZV ZW
	14	Snowy River	Feb	no cloud	-0.67978	0.290938	Inf	-1.61663	0.257066	R ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT ZU ZV ZW
	7	Patersons Terrace	Mar	high cloud	-0.6717	0.324627	Inf	-1.71702	0.373631	M O S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT ZU ZV ZW
	37	Patersons Terrace	Mar	patchy cloud	-0.61458	0.333829	Inf	-1.68954	0.460376	K O S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT ZU ZV ZW
	19	Patersons Terrace	Nov	no cloud	-0.49536	0.317345	Inf	-1.51724	0.526525	ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN ZO ZP ZQ ZR ZS ZT ZU ZV ZW
	5	Patersons Terrace	Jan	high cloud	-0.40148	0.321899	Inf	-1.43803	0.635058	W X Y ZA ZC ZD ZE ZG ZH ZJ ZL ZN ZP ZQ ZR ZS ZT ZU ZV ZW
	3	Patersons Terrace	Feb	high cloud	-0.35706	0.322223	Inf	-1.39465	0.680529	Y ZA ZE ZH ZJ ZQ ZR ZS ZT ZU ZV ZW
	35	Patersons Terrace	Jan	patchy cloud	-0.34437	0.32789	Inf	-1.40021	0.711462	T U ZD ZF ZG ZH ZI ZJ ZM ZN ZO ZP ZR ZS ZT ZU ZV ZW

33	Patersons Terrace	Feb	patchy cloud	-0.29995	0.329172	Inf	-1.35991	0.760016	U ZG ZI ZJ ZO ZP ZS ZT ZU ZV ZW
17	Patersons Terrace	Mar	no cloud	-0.22985	0.295334	Inf	-1.18086	0.721148	ZT ZU ZV ZW
15	Patersons Terrace	Jan	no cloud	0.040357	0.29482	Inf	-0.90899	0.989705	ZU ZW
13	Patersons Terrace	Feb	no cloud	0.084782	0.298587	Inf	-0.87669	1.046258	ZV ZW

Table D.2. Post-hoc pairwise comparisons of p on a 100 m^2 transect at Patersons Terrace and Snowy River in November, December, January, February, March under no cloud, high cloud, patchy cloud and overcast weather conditions. Common letters denote no significant difference at $p < 0.05$.

	Location	Month	Sky	lsmean	SE	df	asymp.LCL	asymp.UCL	group
22	Snowy River	Dec	overcast	-1.74708	0.623267	Inf	-3.75406	0.259892	A
28	Snowy River	Mar	overcast	-1.35284	0.639563	Inf	-3.41229	0.706611	A B
30	Snowy River	Nov	overcast	-1.16303	0.651048	Inf	-3.25946	0.933405	A B C
26	Snowy River	Jan	overcast	-0.58568	0.640774	Inf	-2.64903	1.477665	A B C D E F G H I J K
32	Snowy River	Dec	patchy cloud	-0.55699	0.65891	Inf	-2.67874	1.564756	A B D E G I
2	Snowy River	Dec	high cloud	-0.46419	0.619051	Inf	-2.45759	1.529205	A B C D F G H
21	Patersons Terrace	Dec	overcast	-0.31313	0.605718	Inf	-2.2636	1.637333	A B C D E F J L
38	Snowy River	Mar	patchy cloud	-0.16275	0.656545	Inf	-2.27688	1.951383	A B C D E F G H I J K L M N O P
8	Snowy River	Mar	high cloud	-0.06995	0.647236	Inf	-2.15411	2.014207	A B C D E F G H I J K L M N Q R S T U V
40	Snowy River	Nov	patchy cloud	0.027063	0.67038	Inf	-2.13162	2.185748	A B C D E F G H I J K L M O Q W X Y ZA
27	Patersons Terrace	Mar	overcast	0.081109	0.621111	Inf	-1.91892	2.08114	A B C D E F G H I J K L M Q
10	Snowy River	Nov	high cloud	0.11986	0.684955	Inf	-2.08575	2.325474	A B C D E F G H I J K L M Q T U W X ZB ZC
24	Snowy River	Feb	overcast	0.121144	0.667394	Inf	-2.02792	2.270211	B C D E F G H I J K L M N O P S U W Y ZB ZD ZE ZF ZG

29	Patersons Terrace	Nov	overcast	0.27092	0.637775	Inf	-1.78277	2.324611	A B C D E F G H I J K L M Q T U
12	Snowy River	Dec	no cloud	0.574035	0.575987	Inf	-1.2807	2.428765	B C D E F G H I J K L M N O P S U W Y ZB ZD ZE ZF ZG
36	Snowy River	Jan	patchy cloud	0.604408	0.646211	Inf	-1.47645	2.685263	B C D E F G H I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK
6	Snowy River	Jan	high cloud	0.697205	0.6523	Inf	-1.40326	2.797668	B C D E F G H I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL
25	Patersons Terrace	Jan	overcast	0.848265	0.636393	Inf	-1.20098	2.897507	B C D E F G H I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK
31	Patersons Terrace	Dec	patchy cloud	0.876956	0.680594	Inf	-1.31462	3.068528	A B C D E F G H I J K L M N O P Q R W X Y ZA ZD ZE ZH ZI
18	Snowy River	Mar	no cloud	0.968278	0.556875	Inf	-0.82491	2.761466	D E F G H I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZM
1	Patersons Terrace	Dec	high cloud	0.969753	0.648354	Inf	-1.118	3.05751	A B C D E F G H I J K L M N Q R S T U V W X ZB ZC ZD ZF ZH ZJ
20	Snowy River	Nov	no cloud	1.15809	0.601304	Inf	-0.77816	3.094343	D E F G H I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZM

37	Patersons Terrace	Mar	patchy cloud	1.2712	0.677053	Inf	-0.90897	3.451369	A B C D E F G H I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZM
34	Snowy River	Feb	patchy cloud	1.311235	0.67054	Inf	-0.84796	3.470433	C F H J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZM
7	Patersons Terrace	Mar	high cloud	1.363997	0.67406	Inf	-0.80654	3.534529	A B C D E F G H I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN
4	Snowy River	Feb	high cloud	1.404032	0.646271	Inf	-0.67702	3.485081	E I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZM
39	Patersons Terrace	Nov	patchy cloud	1.461011	0.694921	Inf	-0.7767	3.698717	B C D E F G H I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN
9	Patersons Terrace	Nov	high cloud	1.553808	0.714675	Inf	-0.74751	3.855124	A B C D E F G H I J K L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN
23	Patersons Terrace	Feb	overcast	1.555092	0.66732	Inf	-0.59374	3.703922	G H I K M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZM
16	Snowy River	Jan	no cloud	1.735434	0.576853	Inf	-0.12208	3.59295	L M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN

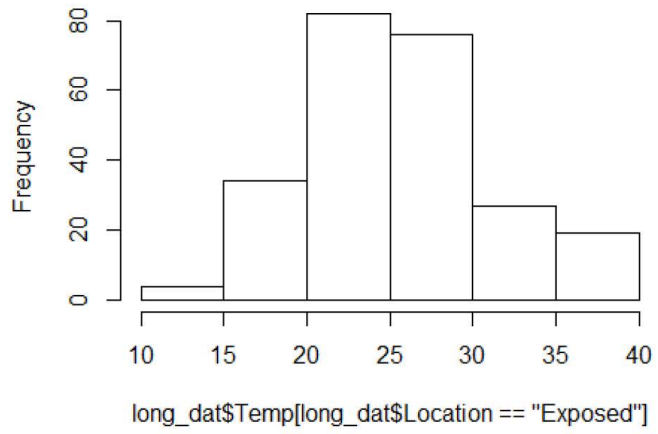
11	Patersons Terrace	Dec	no cloud	2.007983	0.609221	Inf	0.046238	3.969728	K M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZM
35	Patersons Terrace	Jan	patchy cloud	2.038356	0.680151	Inf	-0.15179	4.228503	H K M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN
5	Patersons Terrace	Jan	high cloud	2.131153	0.691813	Inf	-0.09655	4.358852	I K M N O P Q R S T U V W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN
17	Patersons Terrace	Mar	no cloud	2.402226	0.589748	Inf	0.503185	4.301267	W X Y ZA ZB ZC ZD ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN
14	Snowy River	Feb	no cloud	2.442261	0.630078	Inf	0.413353	4.471169	Q R T V X ZA ZC ZH ZI ZJ ZK ZL ZM ZN
19	Patersons Terrace	Nov	no cloud	2.592037	0.636722	Inf	0.541736	4.642339	NP R S V Z D ZE ZF ZG ZH ZI ZJ ZK ZL ZM ZN
33	Patersons Terrace	Feb	patchy cloud	2.745183	0.707204	Inf	0.467923	5.022442	S T U V ZB ZC ZF ZG ZJ ZK ZL ZM ZN
3	Patersons Terrace	Feb	high cloud	2.83798	0.690125	Inf	0.615717	5.060242	O P Y ZA ZE ZG ZI ZK ZL ZM ZN
15	Patersons Terrace	Jan	no cloud	3.169382	0.622992	Inf	1.163291	5.175473	ZM ZN
13	Patersons Terrace	Feb	no cloud	3.876209	0.67665	Inf	1.697335	6.055083	ZL ZN

Appendix E

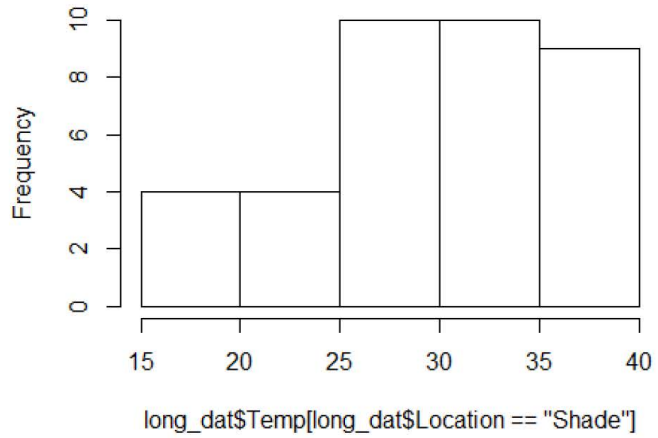
Chapter 3: Data analyses and R code

Chapter 3: Using radio transmitter to reveal habitat use of a Nationally Endangered grasshopper with implications for conservation management

```
# ----------------
# read in the data
# ----------------

long_dat<- read.csv('C:/Users/Jennifer/Desktop/Data
chapters/Transmitter/Temperature and Location
analysis/Temp_Location_data_long.csv')

# test assumptions
var.test(long_dat$Temp[long_dat$Location == "Exposed"],
long_dat$Temp[long_dat$Location == "Shade"])

##
##  F test to compare two variances
##
## data:  long_dat$Temp[long_dat$Location == "Exposed"] and
long_dat$Temp[long_dat$Location == "Shade"]
## F = 0.85293, num df = 241, denom df = 36, p-value = 0.4823
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.4929165 1.3442448
## sample estimates:
## ratio of variances
##          0.8529316

hist(long_dat$Temp[long_dat$Location == "Exposed"], main = NULL)
```




```
hist(long_dat$Temp[long_dat$Location == "Shade"], main = NULL)
```



```r
# check out the means
print(mean(long_dat$Temp[long_dat$Location == "Exposed"]))
```

```
## [1] 25.7595
```

```r
print(mean(long_dat$Temp[long_dat$Location == "Shade"]))
```

```
## [1] 29.38649
```

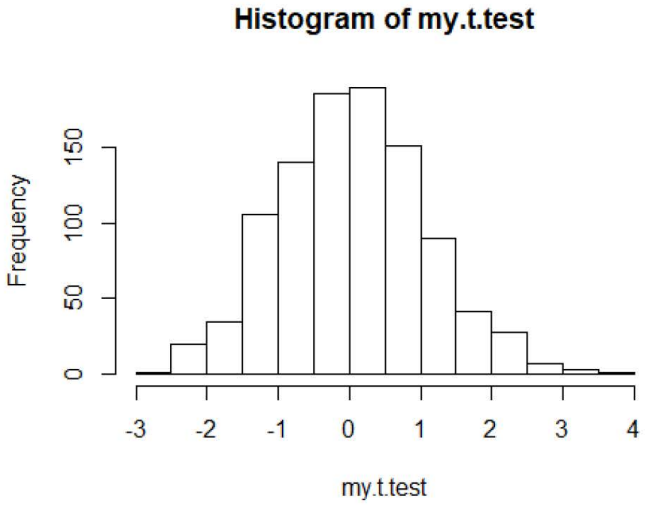
```r
# t-test
t.test(Temp ~ Location, dat = long_dat)
```

```
##
##  Welch Two Sample t-test
##
## data:  Temp by Location
## t = -3.4959, df = 45.885, p-value = 0.00106
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -5.715465 -1.538500
## sample estimates:
## mean in group Exposed   mean in group Shade
##              25.75950              29.38649
```

```r
# -----------------
# Permutation test
# -----------------

set.seed(26092019)

my.t.test <- numeric(999)

for(i in 1:999){
  my.t.test[i] <- t.test(Temp ~ sample(Location), data=long_dat)$statistic
}

summary(my.t.test)
```

```
##     Min.  1st Qu.   Median     Mean  3rd Qu.     Max.
## -2.77658 -0.69217  0.03500  0.04778  0.73312  3.97585
```

```r
# distribution of t-statistics
hist(my.t.test)
```




```
# The t statistic for the observed data set
t.obs <- t.test(Temp ~ Location, data=long_dat)$statistic
t.obs

##        t
## -3.49595

#-----------------------------------
# Cloud cover and grasshopper position
#-----------------------------------

#For Ohau River

Ohaucc <- as.matrix(c(44,5,7,3,22,1,9,0,0,1,0,0,15,1,0,1,2,1,1,2,1,0,2,1))
dim(Ohaucc) <- c(6,4)
Ohaucc

##      [,1] [,2] [,3] [,4]
## [1,]   44    9   15    1
## [2,]    5    0    1    2
## [3,]    7    0    0    1
## [4,]    3    1    1    0
## [5,]   22    0    2    2
## [6,]    1    0    1    1

fisher.test(Ohaucc)
```

```
##
##  Fisher's Exact Test for Count Data
##
## data:  Ohaucc
## p-value = 0.02451
## alternative hypothesis: two.sided
```

```
#For Patersons Terrace

Pattcc <- as.matrix(c(65,27,19,47,7,1,0,0,0,0,6,0,0,0,0,0,1,1,3,1))
dim(Pattcc) <- c(5,4)
Pattcc
```

```
##      [,1] [,2] [,3] [,4]
## [1,]   65    1    6    0
## [2,]   27    0    0    1
## [3,]   19    0    0    1
## [4,]   47    0    0    3
## [5,]    7    0    0    1
```

```
fisher.test(Pattcc)
```

```
##
##  Fisher's Exact Test for Count Data
##
## data:  Pattcc
## p-value = 0.07171
## alternative hypothesis: two.sided
```

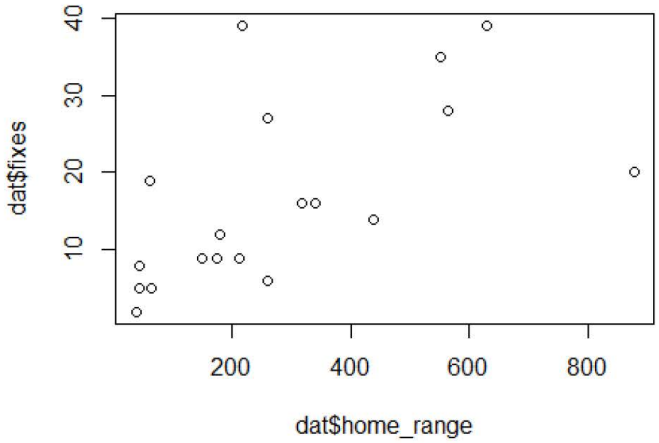
```
#------------------------------
# Wind and grasshopper location
#------------------------------

#For Ohau River

Ohauwind <- as.matrix(c(18,59,5,0,10,0,2,16,2,1,3,3))
dim(Ohauwind) <- c(3,4)
Ohauwind
```

```
##      [,1] [,2] [,3] [,4]
## [1,]   18    0    2    1
## [2,]   59   10   16    3
## [3,]    5    0    2    3
```

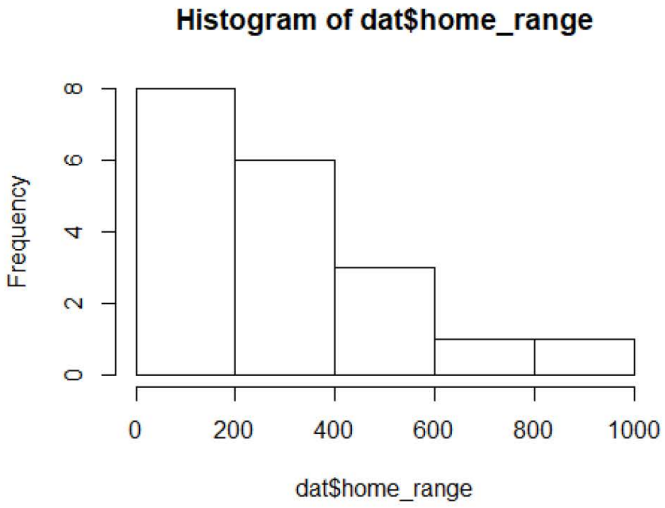
```
fisher.test(Ohauwind)
```

```
##
##  Fisher's Exact Test for Count Data
##
## data:  Ohauwind
```

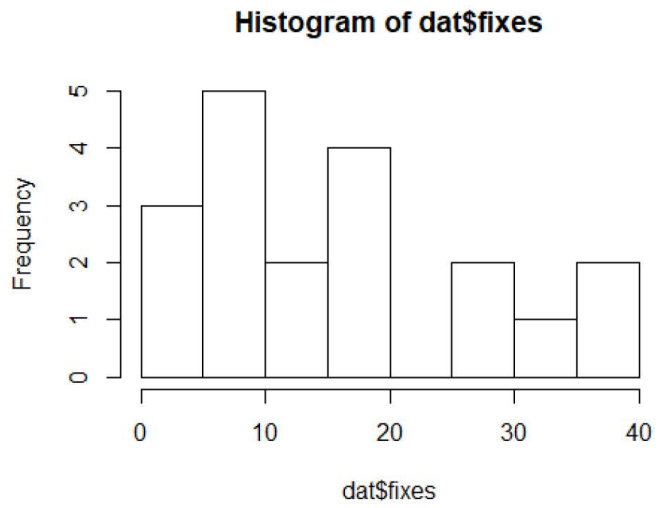
```
## p-value = 0.03714
## alternative hypothesis: two.sided

#For Patersons Terrace

Pattwind <- as.matrix(c(47,53,65,0,1,0,4,1,1,1,2,3))
dim(Pattwind) <- c(3,4)
Pattwind

##      [,1] [,2] [,3] [,4]
## [1,]   47    0    4    1
## [2,]   53    1    1    2
## [3,]   65    0    1    3

fisher.test(Pattwind)

##
##  Fisher's Exact Test for Count Data
##
## data:  Pattwind
## p-value = 0.4117
## alternative hypothesis: two.sided

# ---------------------------------------
# Home range size and the number of fixes
# ---------------------------------------

# --------------
# input the data
# --------------

home_range <-
c(317.97,210.45,258.64,43.78,173.95,178.19,60.05,564.43,877.91,340.82,436.74,
628.14,148.73,62.61,36.78,44.07,550.43,259.79,217.15)

fixes <-c(16,9,6,8,9,12,19,28,20,16,14,39,9,5,2,5,35,27,39)

dat <- data.frame(home_range, fixes)

# ----------------
# correlation test
# ----------------

#plot data
plot(dat$home_range, dat$fixes)
```




```
#check normality of Home range
hist(dat$home_range)
```

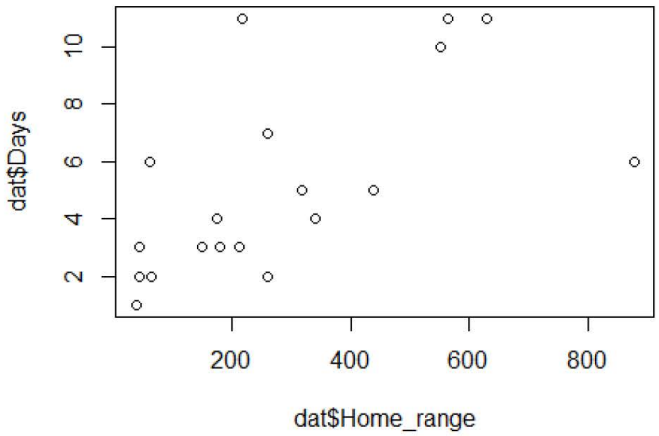


```
#check normality of fixes
hist(dat$fixes)
```

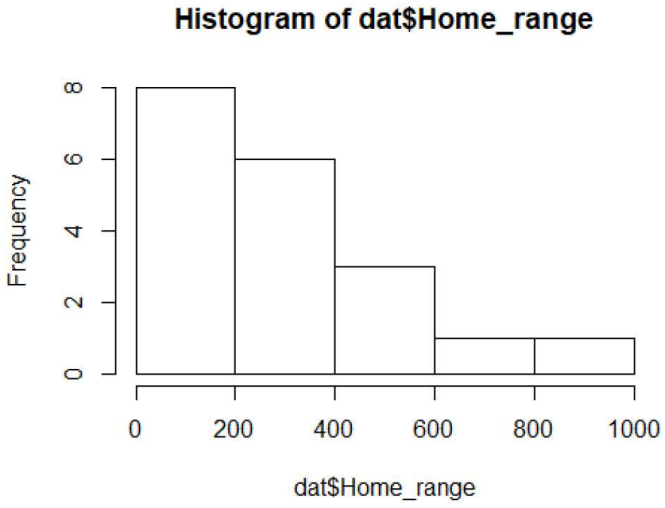



```
#correlation test for non-normal data
cor.test(dat$home_range, dat$fixes, method="kendall")

##
##  Kendall's rank correlation tau
##
## data:  dat$home_range and dat$fixes
## z = 3.3373, p-value = 0.000846
## alternative hypothesis: true tau is not equal to 0
## sample estimates:
##       tau
## 0.5655664

# ------------------------------------------
# Home range size and number of tracking days
# ------------------------------------------

# --------------
# input the data
# --------------

Location <- as.factor(rep(c("Ohau", "Patersons"), times = c(9,10)))

Home_range <-
```

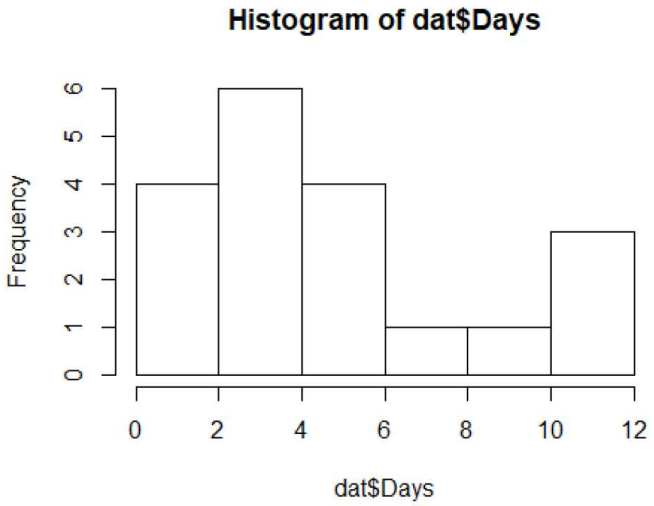
```
c(317.97,210.45,258.64,43.78,173.95,178.19,60.05,564.43,877.91,340.82,436.74,
628.14,148.73,62.61,36.78,44.07,550.43,259.79,217.15)

Days <-c(5,3,2,3,4,3,6,11,6,4,5,11,3,2,1,2,10,7,11)

dat <- data.frame(Location, Home_range, Days)
dat$log_homerange <- log(dat$Home_range)

# ----------------
# correlation test
# ----------------

#plot data
plot(dat$Home_range, dat$Days)
```



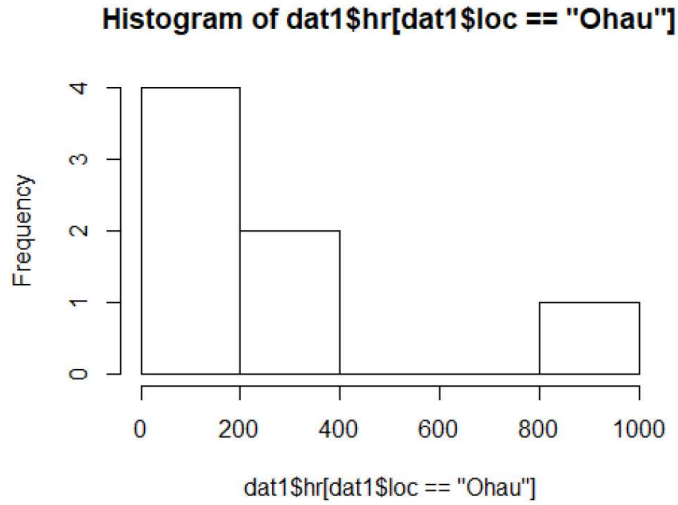

```
#check normality of Home range
hist(dat$Home_range)
```



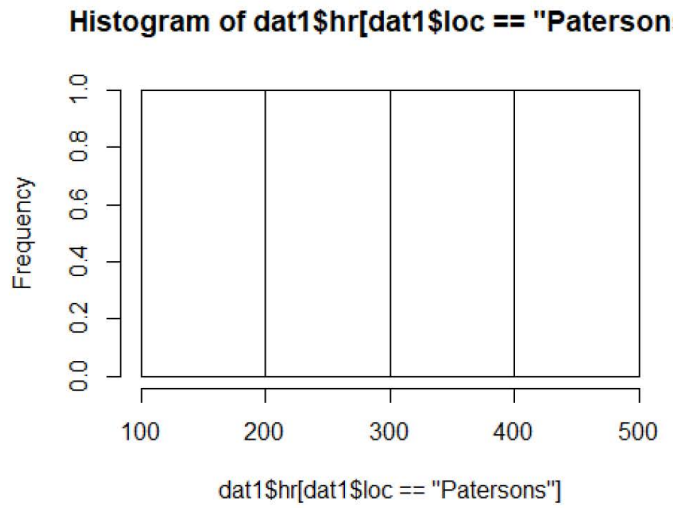

```
#check normality of days
hist(dat$Days)
```



```r
#correlation test for non-normal data
cor.test(dat$Home_range, dat$Days, method="kendall")

##
##  Kendall's rank correlation tau
##
## data:  dat$Home_range and dat$Days
## z = 3.186, p-value = 0.001443
## alternative hypothesis: true tau is not equal to 0
## sample estimates:
##       tau
## 0.5510388

# ------------------------------------------------
# home range at different locations for days 3 to 7
# ------------------------------------------------

# -------------
# read in data
# -------------

loc <- c("Ohau","Ohau","Ohau","Ohau","Ohau","Ohau","Ohau",
"Patersons","Patersons","Patersons","Patersons")
hr <- c(317.97, 210.45, 43.78, 173.95, 178.19, 60.05, 877.91, 340.82, 436.74,
148.73, 259.79)

dat1 <- data.frame(loc, hr)

#---------
# t -test
# --------

# check assumptions
var.test(hr ~ loc, data = dat1)

##
##  F test to compare two variances
##
## data:  hr by loc
## F = 5.4468, num df = 6, denom df = 3, p-value = 0.1924
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##   0.3696602 35.9425940
## sample estimates:
## ratio of variances
##           5.446839

hist(dat1$hr[dat1$loc == "Ohau"])
```




```
hist(dat1$hr[dat1$loc == "Patersons"])
```



```r
# check out the means
print(mean(dat1$hr[dat1$loc == "Ohau"]))
```

```
## [1] 266.0429
```

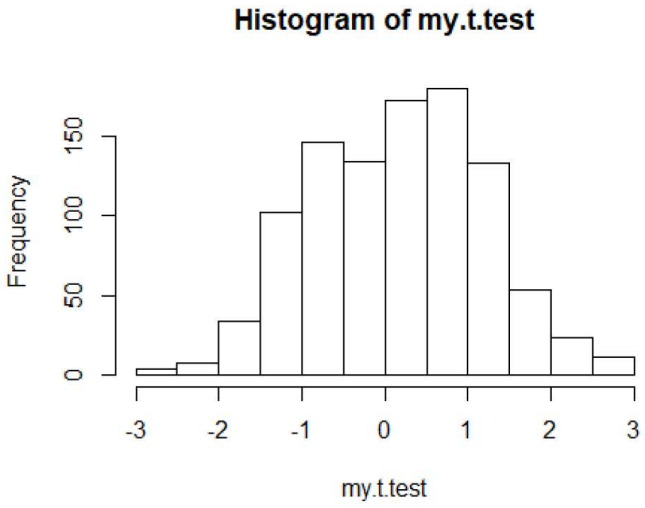
```r
print(mean(dat1$hr[dat1$loc == "Patersons"]))
```

```
## [1] 296.52
```

```r
# ------------------
# Permutation test
# ------------------

my.t.test <- numeric(999)

for(i in 1:999){
  my.t.test[i] <- t.test(hr ~ sample(loc), data=dat1)$statistic
}

summary(my.t.test)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
## -2.5356 -0.6456  0.2739  0.1636  0.9070  2.8031
```

```r
# distribution of t-statistics
hist(my.t.test)
```



```r
# The t-statistic for the observed data set
t.obs <- t.test(hr ~ loc, data=dat1)$statistic

t.obs
```

```
##          t
## -0.2459239
```

```r
# ----------------------------------------------------------
# model home range size and tracking days ALL GRASSHOPPERS
# ----------------------------------------------------------

library(lme4)
```

```
## Loading required package: Matrix
```

```r
library(lmerTest)
```

```
##
## Attaching package: 'lmerTest'
```

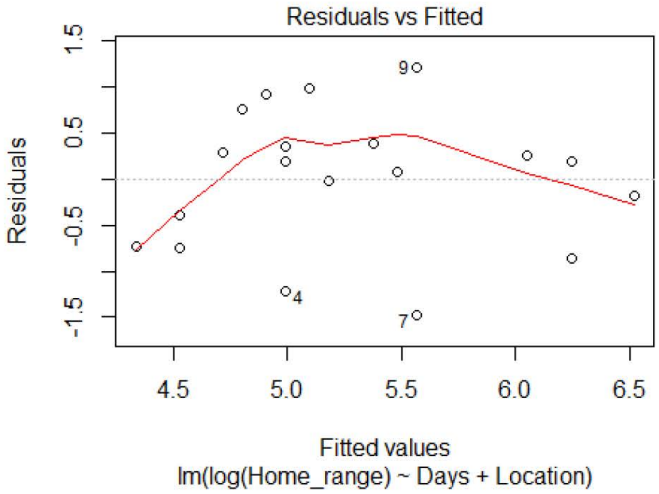
```
## The following object is masked from 'package:lme4':
##
##     lmer
```

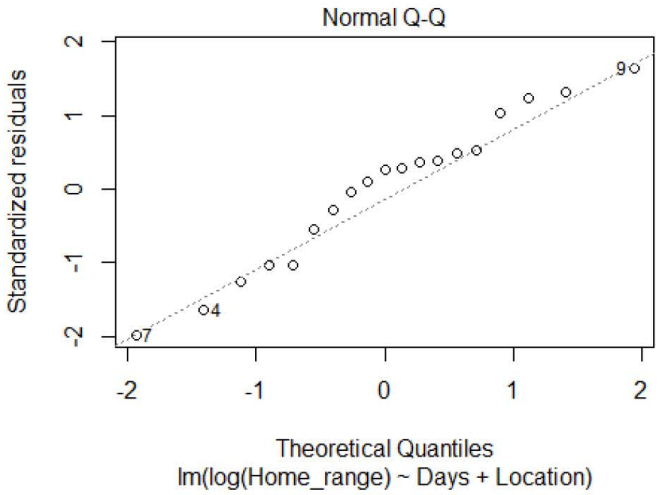
```
## The following object is masked from 'package:stats':
##
##     step
```

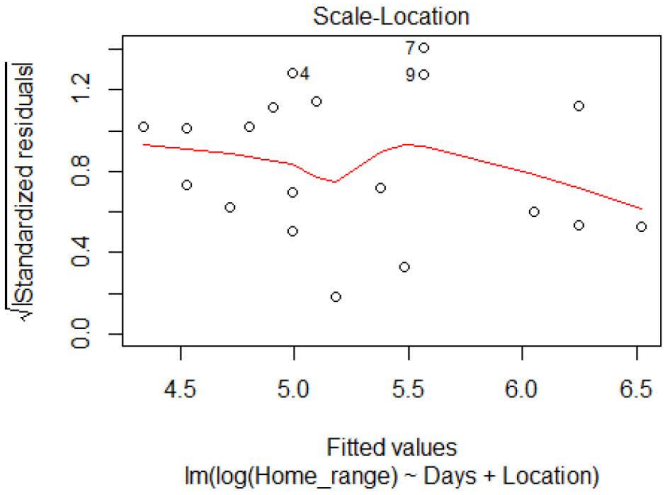
```r
mod <- lm(log(Home_range) ~ Days + Location, data = dat)
summary(mod)
```

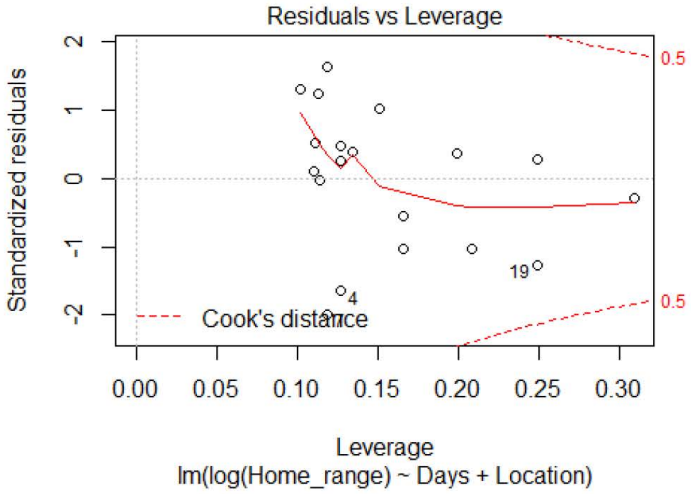
```
##
## Call:
## lm(formula = log(Home_range) ~ Days + Location, data = dat)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.4711 -0.5597  0.1894  0.3712  1.2113
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        4.42064    0.37727  11.718  2.9e-09 ***
## Days               0.19094    0.05649   3.380  0.00382 **
## LocationPatersons -0.27644    0.36630  -0.755  0.46141
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.7908 on 16 degrees of freedom
## Multiple R-squared:  0.4189, Adjusted R-squared:  0.3462
## F-statistic: 5.766 on 2 and 16 DF,  p-value: 0.01301
```

```
#check model fit
plot(mod)
```



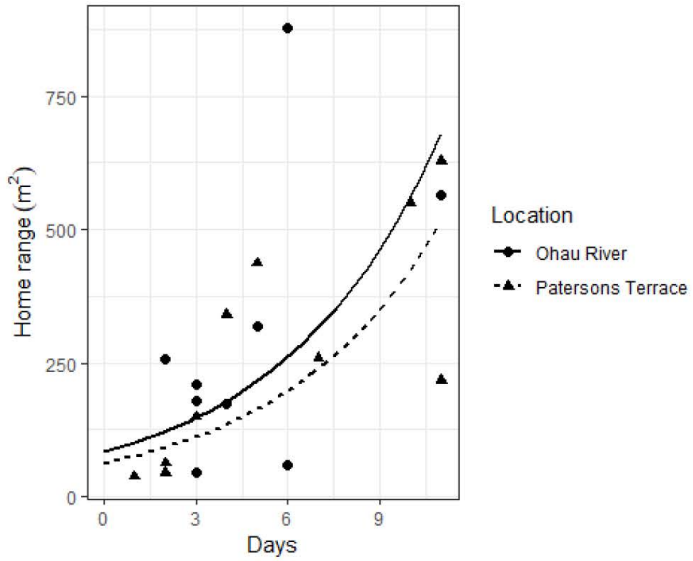







```
# --------------
# plot the model
# --------------
```

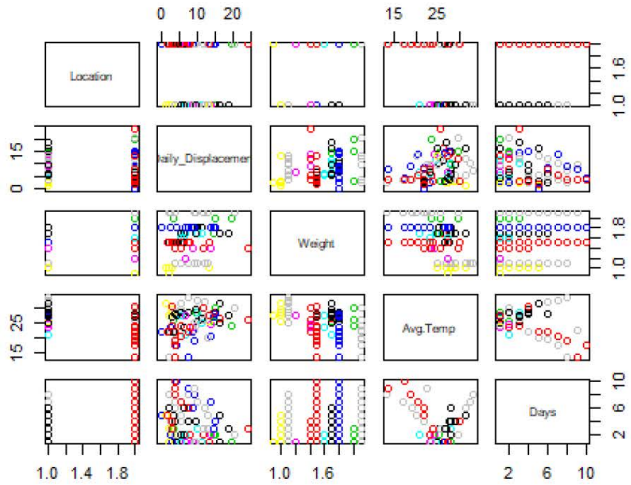
```
newdata <- expand.grid(Days=seq(0,11, length=33),
                       Location=levels(dat$Location))

newdata$pred_Home_range <- predict(mod, newdata=newdata)

#back transform data
newdata$predicted <- exp(newdata$pred_Home_range)

#rename levels
levels(dat$Location) <- c("Ohau River", "Patersons Terrace")
levels(newdata$Location) <- c("Ohau River", "Patersons Terrace")

# plot

library(ggplot2)

ggplot(dat, aes(y=Home_range, x=Days)) +
  geom_point(aes(shape = Location), size = 2) +
  geom_line(data=newdata, aes(y=predicted, linetype = Location), size = 1) +
  #facet_wrap(~Location) +
  theme_bw() +
  ylab("Home range" ~(m^2)) +
  xlab("Days")
```



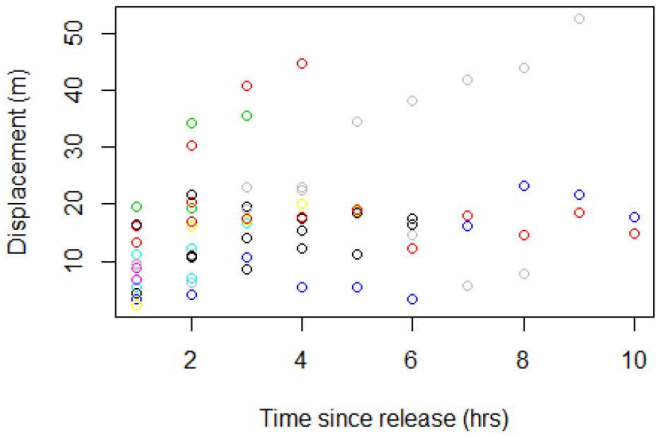

```
# ------------------
# Relocation model
```

```
# ------------------

# ------
# Generate the mean temperatures for each tracking period from the data
collected off the hoboware loggers
# ------

# Patersons Terrace data

Patt_tempdat <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Weather
Data/Pattersons_GroundTemp_May2018.csv', header = TRUE)

#Temperature data: Make new column with date and time in correct format
Patt_tempdat$Date_Time <- as.POSIXct(paste(Patt_tempdat$Date,
Patt_tempdat$Time), format = "%d/%m/%y %H:%M:%S")

# Ohau River data

Ohau_tempdat <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Weather
Data/Aviary_GroundTemp_June2018.csv', header = TRUE)

#Temperature data: Make new column with date and time in correct format
Ohau_tempdat$Date_Time <- as.POSIXct(paste(Ohau_tempdat$Date,
Ohau_tempdat$Time), format = "%d/%m/%Y %H:%M:%S")

# ---------
# Time Data
# ---------

timesdat <- read.csv('C:/Users/Jennifer/Desktop/Data
Chapters/Transmitter/24hr_Turning angles prepared for analysis.csv')

#Time data: make sure "start" and "end" times in correct format for R
timesdat$Date_Time_Start1 <- as.POSIXct(paste(timesdat$Date_Time_Start),
format = "%d/%m/%Y %H:%M")

timesdat$Date_Time_End1 <- as.POSIXct(paste(timesdat$Date_Time_End), format =
"%d/%m/%Y %H:%M")

#get rid of empty column
timesdat$Avg_Temp <- NULL

#get rid of rows with "NA"
timesdat <- na.omit(timesdat)

# create separate files for Ohau and Patersons locations
X <- split(timesdat, timesdat$Location)
Ohau <- X[[1]]
Pattersons <- X[[2]]
```

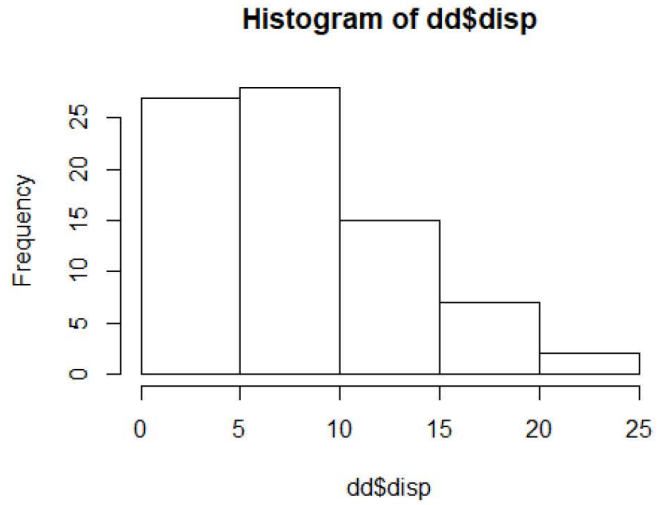
```
# -----------
# get the avg temps for the times at each location
# -----------

for (i in 1:nrow(Ohau)){
  x <- Ohau_tempdat[which(Ohau_tempdat$Date_Time >= Ohau$Date_Time_Start1[i]
& Ohau_tempdat$Date_Time <= Ohau$Date_Time_End1[i]),]
  Ohau$Avg.Temp[i] <- mean(x$Temp)
}

for (i in 1:nrow(Pattersons)){
  x <- Patt_tempdat[which(Patt_tempdat$Date_Time >=
Pattersons$Date_Time_Start1[i] & Patt_tempdat$Date_Time <=
Pattersons$Date_Time_End1[i]),]
  Pattersons$Avg.Temp[i] <- mean(x$Temp)
}

#merge back into a single file
Z <- rbind(Ohau, Pattersons)

#export as .csv
#write.csv(Z, file = 'C:/Users/Jennifer/Desktop/Data
Chapters/Transmitter/24hrly displacement w avg temp.csv')

# ---------------
# Use the data to write model to analyse displacement distances
# ---------------

dailydisp <- Z
#dailydisp <- read.csv('C:/Users/Jennifer/Desktop/Data
Chapters/Transmitter/24hrly displacement w avg temp.csv')

# add new column which contains a count of "days released"
dailydisp$Days <- sequence(rle(as.character(dailydisp$ID))$lengths)

#add new coloumn with the the ratio or transmitter weight and grasshopper
body weight
dailydisp$Ratio <- 0.22/dailydisp$Weight

#get rid of rows with "NA"
dailydisp <- na.omit(dailydisp)

#add column with temp squared
dailydisp$temp_sq <- dailydisp$Avg.Temp*dailydisp$Avg.Temp

# scatterplot
plot(dailydisp[,c(2,9,11,14,15)], col=dailydisp$ID) #plot distance vs weight
vs temp vs days vs location
```



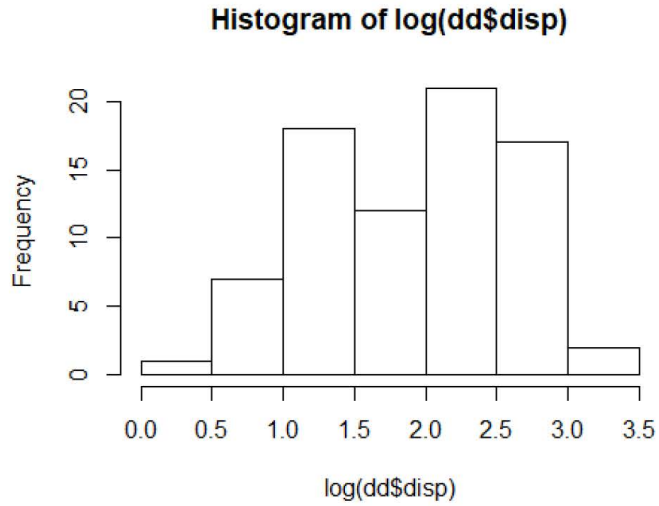

```
plot(dailydisp$Days, dailydisp$Displacement,
     xlab="Time since release (hrs)",
     ylab="Displacement (m)",
     main="",
     col= dailydisp$ID) #make sure the colours represent species
```



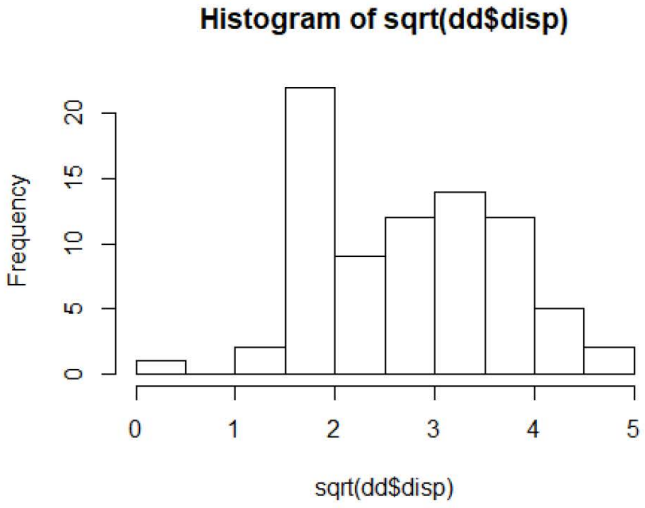

```
# Subset dataframe ----

# columns of interest (weight, avg. temp, displacement, ID, location etc.)

dd <- dailydisp
dd$disp <- dd$Daily_Displacement
dd$Daily_Displacement <- NULL

# Model testing ----

hist(dd$disp) # skewed data
```




```
hist(log(dd$disp))
```

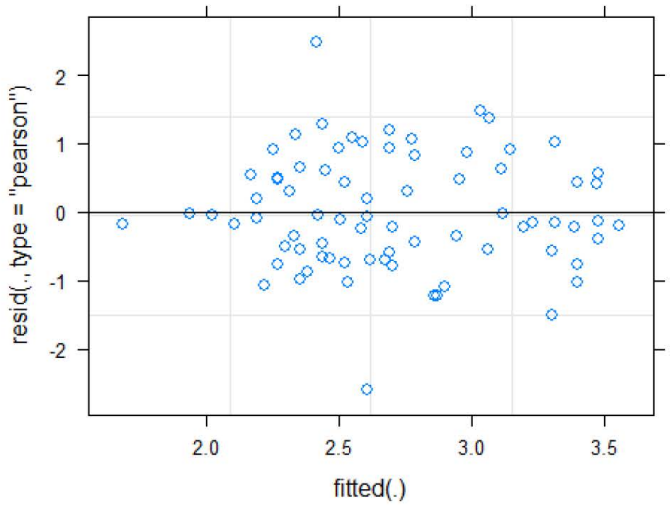



```
hist(sqrt(dd$disp))
```

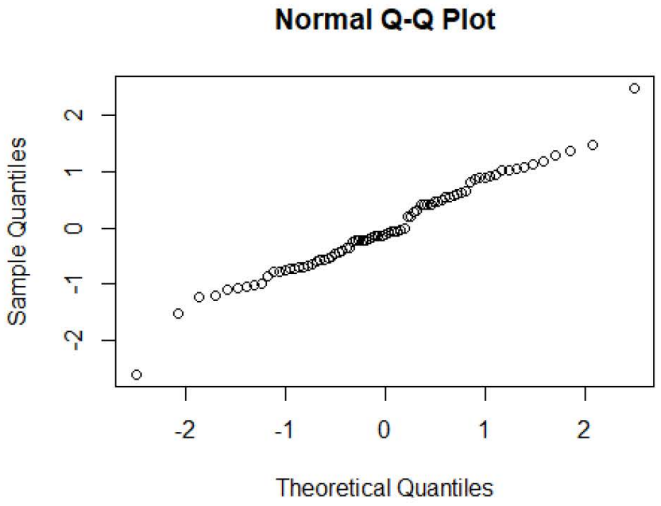



```
# check out the means
print(mean(dd$disp[dd$Location == "Pattersons"]))

## [1] 7.417391

print(mean(dd$disp[dd$Location == "Ohau"]))

## [1] 9.587879

library(lme4)
library(lmerTest)


model1 <- lmer(sqrt(disp)  ~ Days + Ratio + Avg.Temp + Location + (1|ID),
data = dd)

## singular fit

model2 <- lmer(sqrt(disp)  ~ Days + Ratio + Avg.Temp + (1|ID), data = dd)

model3 <- lmer(sqrt(disp)  ~ Days + Ratio + Location + (1|ID), data = dd)

## singular fit

# Model diagnostics ----

anova(model1, model2) #Location is  important
```

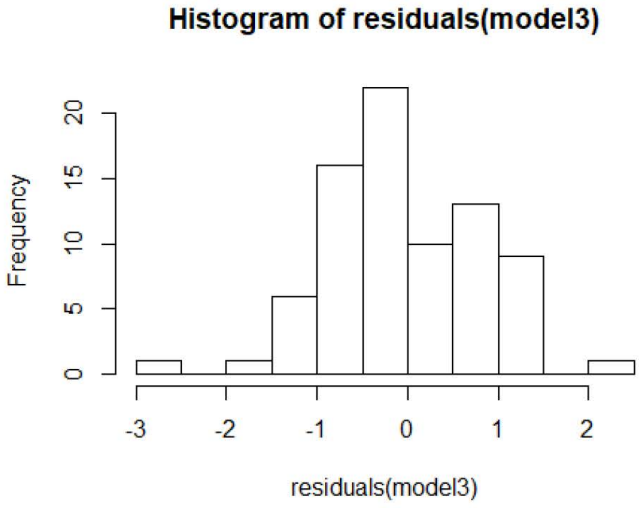
```
## refitting model(s) with ML (instead of REML)

## Data: dd
## Models:
## model2: sqrt(disp) ~ Days + Ratio + Avg.Temp + (1 | ID)
## model1: sqrt(disp) ~ Days + Ratio + Avg.Temp + Location + (1 | ID)
##        Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## model2  6 206.06 220.28 -97.031   194.06
## model1  7 203.65 220.23 -94.823   189.65 4.4153      1    0.03562 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

anova(model1, model3) #Avg_temp is  not important

## refitting model(s) with ML (instead of REML)

## Data: dd
## Models:
## model3: sqrt(disp) ~ Days + Ratio + Location + (1 | ID)
## model1: sqrt(disp) ~ Days + Ratio + Avg.Temp + Location + (1 | ID)
##        Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## model3  6 205.34 219.56 -96.671   193.34
## model1  7 203.65 220.23 -94.823   189.65 3.6967      1    0.05452 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

anova(model2, model3)

## refitting model(s) with ML (instead of REML)

## Data: dd
## Models:
## model2: sqrt(disp) ~ Days + Ratio + Avg.Temp + (1 | ID)
## model3: sqrt(disp) ~ Days + Ratio + Location + (1 | ID)
##        Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## model2  6 206.06 220.28 -97.031   194.06
## model3  6 205.34 219.56 -96.671   193.34 0.7187      0  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

AIC(model1, model2, model3) #model 3 lowest AIC

##        df      AIC
## model1  7 214.1614
## model2  6 215.1776
## model3  6 210.3939

#check model fit
plot(model3)
```




```
qqnorm(residuals(model3))
```

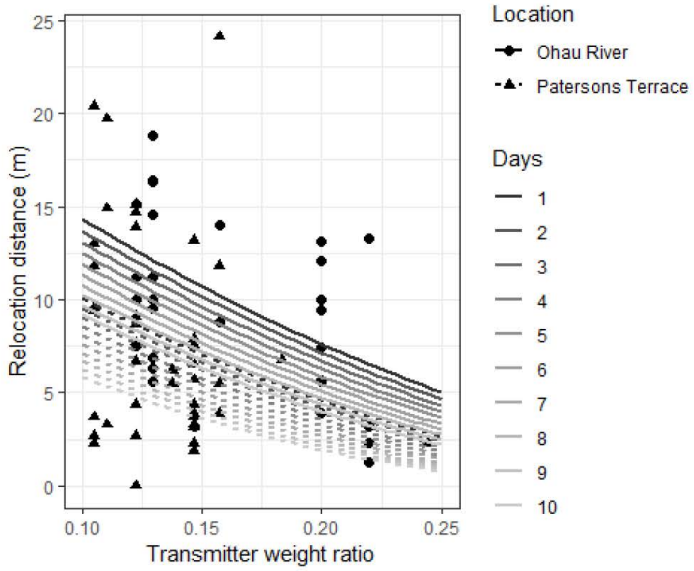



```
hist(residuals(model3))
```




```
# view summary
summary(model3)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: sqrt(disp) ~ Days + Ratio + Location + (1 | ID)
##    Data: dd
##
## REML criterion at convergence: 198.4
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.0895 -0.6873 -0.1418  0.6654  2.9512
##
## Random effects:
##  Groups   Name        Variance  Std.Dev.
##  ID       (Intercept) 8.659e-16 2.943e-08
##  Residual             7.128e-01 8.443e-01
## Number of obs: 79, groups:  ID, 18
##
## Fixed effects:
##                    Estimate Std. Error        df t value Pr(>|t|)
## (Intercept)         4.89216    0.52032  75.00000   9.402 2.57e-14 ***
## Days               -0.08360    0.03885  75.00000  -2.152  0.03463 *
## Ratio             -10.25297    3.00464  75.00000  -3.412  0.00104 **
## LocationPattersons  -0.61266    0.21554  75.00000  -2.842  0.00576 **
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) Days   Ratio
## Days        -0.223
## Ratio       -0.933  0.001
## LctnPttrsns -0.496 -0.236  0.381
## convergence code: 0
## singular fit

# --------------
# plot the model
# --------------

newdata <- expand.grid(Days= seq(1,10),
                       Ratio = seq(0.1, 0.25, length = 40),
                       Location=levels(dd$Location),
                       ID = levels(dd$ID))

newdata$pred_disp <- predict(model3, newdata=newdata)

# back transfrom data
newdata$predicted <- (newdata$pred_disp)^2

# rename levels
levels(dd$Location) <- c("Ohau River", "Patersons Terrace")
levels(newdata$Location) <- c("Ohau River", "Patersons Terrace")

# plot

library(ggplot2)

newdata$Days <- as.factor(as.integer(newdata$Days))

ggplot(dd, aes(y=disp, x=Ratio)) +
  geom_point(aes(shape=Location), size = 2) +
  geom_line(data=newdata, aes(y=predicted, colour = Days, linetype =
Location), size = 1) +
  #facet_wrap(~Location) +
  theme_bw() +
  ylab("Relocation distance (m)") +
  xlab("Transmitter weight ratio") +
  scale_color_grey()
```




```
# ------------------------
# Turning angle analyses
# ------------------------

# ------------
# Prepare data
# ------------

library(adehabitatLT)

## Loading required package: sp

## Loading required package: ade4

## Loading required package: adehabitatMA

## Loading required package: CircStats

## Loading required package: MASS

## Loading required package: boot

locdat <- read.csv("C:/Users/Jennifer/Desktop/Data
Chapters/Transmitter/24hr_Turning angles prepared for analysis.csv")

#Convert date and time data for correct format
da <- as.character(locdat$Date_Time_Start)
da <-as.POSIXct(strptime(as.character(locdat$Date_Time_Start), format =
```

```
"%d/%m/%Y %H:%M"))
head(da)

## [1] "2017-11-18 09:00:00 NZDT" "2017-11-19 08:15:00 NZDT"
## [3] "2017-11-20 08:00:00 NZDT" "2017-11-21 08:00:00 NZDT"
## [5] "2017-11-22 08:00:00 NZDT" "2017-11-18 09:00:00 NZDT"

#Store the movement data as an object class of ltraj
puech <- as.ltraj(xy = locdat[,c("Lat","Long")], date = da, id = locdat$ID,
burst = locdat$ID)

# -------------
# Get the data for Ohau + Patersons turning angles
# -------------

#combine the Ohau individuals into one data set
Ohau_Data <-
as.data.frame(rbind(puech[[1]],puech[[2]],puech[[3]],puech[[4]],puech[[5]],pu
ech[[6]],puech[[7]],puech[[8]],puech[[9]]))

#combine the Patersons individuals into one data set
Patt_Data <-
as.data.frame(rbind(puech[[10]],puech[[11]],puech[[12]],puech[[13]],puech[[14
]],puech[[15]],puech[[16]],puech[[17]],puech[[18]]))

# ----------------------
# Relative turning angle
# ----------------------

# a function to round the bearings to the nearest x value (as in the compass
is divided into x sections (instead of infinite))
mround <- function(x,base){
  base*round(x/base)
}

#
# OHAU
#

# get a list of the radians in a new data set
Ohau1 <- data.frame(Rel.angle = as.numeric(Ohau_Data$rel.angle))

# get rid of NAs
Ohau1 <- na.omit(Ohau1)

# convert the negative radians into positive ones
Ohau1$Rel.angle[Ohau1$Rel.angle<0] <- 2*pi +
Ohau1$Rel.angle[Ohau1$Rel.angle<0]
```

```r
# calculate the mean of the cosines and sines:
sum.cos = sum(cos(Ohau1$Rel.angle))
sum.sin = sum(sin(Ohau1$Rel.angle))

# take the arctangent:
x = atan2(sum.sin, sum.cos)

# convert to degrees:
x.deg = x * (180 / pi)

# print
x.deg
```

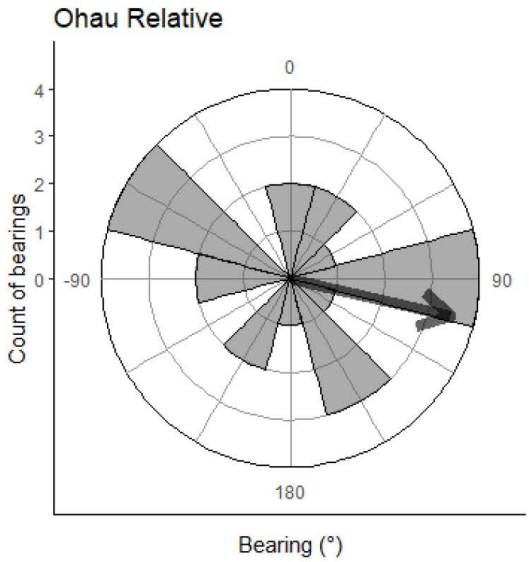
```
## [1] 112.0541
```

```r
## Create plot

# convert the bearing from a radian for compass bearing
Ohau1$bearing <- Ohau1$Rel.angle*(180/pi)

# choose bin size (degrees/bin)
deg <- 30

# define the range of each bin
dir.breaks <- seq(0-(deg/2), 360+(deg/2), deg)

#generate a factor variable, exchanging the directions with the ranges

# assign each direction to a bin range
dir.binned <- cut(Ohau1$bearing,
                  breaks = dir.breaks,
                  ordered_result = TRUE)

# generate labels
dir.labels <- as.character(c(seq(0, 360-deg, by = deg), 0))

# replace ranges with bin labels
levels(dir.binned) <- dir.labels

# Assign bin names to the original data set
Ohau1$dir.binned <- dir.binned

# check what values are needed for the ylab
summary(dir.binned)
```

```
##   0  30  60  90 120 150 180 210 240 270 300 330
##   2   2   1   4   1   3   1   2   0   2   4   0
```

```r
# Prep the plot
thm <- theme_bw() +
```

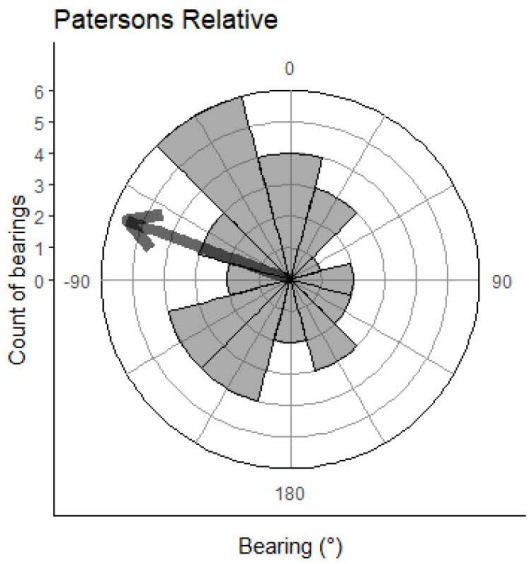
```
  theme(axis.text.x = element_text(size=8, face = "plain"),
        axis.text.y = element_text(size=8, face = "plain"),
        #axis.title.x = element_blank(),
        axis.title.y = element_text(face = "plain", hjust = 0.9, vjust =
1.2),
        panel.border = element_blank(),
        panel.grid  = element_blank())

# initialise the plot
plt.dirrose <- ggplot() +
  # add a series of horizontal lines
  geom_hline(yintercept = seq(0, 4, by = 1), colour = "grey60", size = 0.3) +
  # add a darker horizontal line as the top border
  geom_hline(yintercept = 4, colour = "black", size = 0.5) +
  # We want 12 vertical lines representing the centers of the 30° ranges
  geom_vline(xintercept = c(seq(1,12,1)), colour = "grey60", size = 0.3) +
  # draw histogram bars
  geom_bar(data = Ohau1, aes(x = dir.binned), width = 1, colour="black", size
= 0.3, alpha=0.5) +
  # Add the x-axis labels
  scale_x_discrete( drop = FALSE, labels = c(0, "", "", 90, "", "", 180, "",
"", "-90", "", "")) +
  # Add the y-axis labels
  scale_y_continuous(limits = c(0, 4), expand = c(0, 0),
                     breaks = c(0, 1, 2, 3, 4),
                     labels = c(0, 1, 2, 3, 4)) +
  # Add the axis titles
  labs(x = 'Bearing (°)', y = 'Count of bearings', title = "Ohau Relative") +
  # Add an arrow to point out the mean turning angle
  annotate("segment", x = 4.45, xend = 4.45, y = 0, yend = 3.5, colour =
"black", size=3, alpha=0.6, arrow=arrow()) +
  # wrap the histogram into a windrose
  coord_polar(start = -(deg/2)*(pi/180)) +
  # apply theme
  theme_classic()

# draw the plot
plt.dirrose
```




```
#
# PATERSONS
#

# get a list of the radians in a new data set
Pat1 <- data.frame(Rel.angle = as.numeric(Patt_Data$rel.angle))

# get rid of NAs
Pat1 <- na.omit(Pat1)

# convert the negative radians into positive ones
Pat1$Rel.angle[Pat1$Rel.angle<0] <- 2*pi + Pat1$Rel.angle[Pat1$Rel.angle<0]

# Calculate the mean of the cosines and sines
sum.cos = sum(cos(Pat1$Rel.angle))
sum.sin = sum(sin(Pat1$Rel.angle))

# Take the arctangent
x = atan2(sum.sin, sum.cos)

# Convert to degrees
x.deg = x * (180 / pi)

# Print
x.deg
```

```
## [1] -70.04017

## Create Plot

# convert the bearing from a radian for compass bearing
Pat1$bearing <- Pat1$Rel.angle*(180/pi)

# choose bin size (degrees/bin)
deg <- 30

# define the range of each bin
dir.breaks <- seq(0-(deg/2), 360+(deg/2), deg)

# generate a factor variable, exchanging the directions with the ranges

# assign each direction to a bin range
dir.binned <- cut(Pat1$bearing,
                  breaks = dir.breaks,
                  ordered_result = TRUE)

# generate labels
dir.labels <- as.character(c(seq(0, 360-deg, by = deg), 0))

# replace ranges with bin labels
levels(dir.binned) <- dir.labels

# Assign bin names to the original data set
Pat1$dir.binned <- dir.binned

# check what values are needed for the ylab
summary(dir.binned)

##   0  30  60  90 120 150 180 210 240 270 300 330
##   4   3   1   2   2   3   2   4   4   2   3   6

# Prep the plot
thm <- theme_bw() +
  theme(axis.text.x = element_text(size=8, face = "plain"),
        axis.text.y = element_text(size=8, face = "plain"),
        #axis.title.x = element_blank(),
        axis.title.y = element_text(face = "plain", hjust = 0.9, vjust =
1.2),
        panel.border = element_blank(),
        panel.grid  = element_blank())

# initialise the plot
plt.dirrose <- ggplot() +
  # add a series of horizontal lines
  geom_hline(yintercept = seq(0, 6, by = 1), colour = "grey60", size = 0.3) +
  # add a darker horizontal line as the top border
```

```
  geom_hline(yintercept = 6, colour = "black", size = 0.5) +
  # We want 12 vertical lines representing the centers of the 30° ranges
  geom_vline(xintercept = c(seq(1,12,1)), colour = "grey60", size = 0.3) +
  # draw histogram bars
  geom_bar(data = Pat1, aes(x = dir.binned), width = 1, colour="black", size
= 0.3, alpha=0.5) +
  # Add the x-axis labels
  scale_x_discrete( drop = FALSE, labels = c(0, "", "", 90, "", "", 180, "",
"", "-90", "", "")) +
  # Add the y-axis labels
  scale_y_continuous(limits = c(0, 6), expand = c(0, 0),
                     breaks = c(0, 1, 2, 3, 4, 5, 6),
                     labels = c(0, 1, 2, 3, 4, 5, 6)) +
  # Add the axis titles
  labs(x = 'Bearing (°)', y = 'Count of bearings', title = "Patersons
Relative") +
  #Add an arrow to point out the mean turning angle
  annotate("segment", x = 10.66, xend = 10.66, y = 0, yend = 5.5, colour =
"black", size=3, alpha=0.6, arrow=arrow()) +
  # wrap the histogram into a windrose
  coord_polar(start = -(deg/2)*(pi/180)) +
  # apply theme
  theme_classic()

# draw the plot
plt.dirrose
```



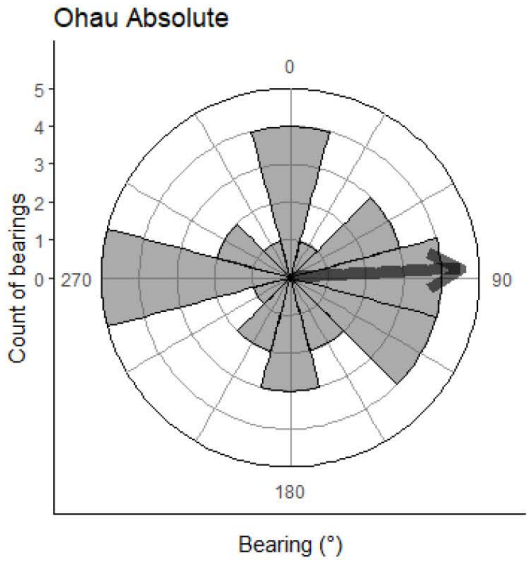
```r
# ----------------------
# Absolute turning angle
# ----------------------

#
# OHAU
#

#get a list of the radians in a new data set
Ohau2 <- data.frame(Abs.angle = as.numeric(Ohau_Data$abs.angle))

#get rid of NAs
Ohau2 <- na.omit(Ohau2)

#convert the negative radians into positive ones
Ohau2$Abs.angle[Ohau2$Abs.angle<0] <- 2*pi +
Ohau2$Abs.angle[Ohau2$Abs.angle<0]

#Calculate the mean of the cosines and sines:
sum.cos = sum(cos(Ohau2$Abs.angle))
sum.sin = sum(sin(Ohau2$Abs.angle))

#Take the arctangent:
x = atan2(sum.sin, sum.cos)

#Convert to degrees:
x.deg = x * (180 / pi)

#Print
x.deg
```

```
## [1] 88.2812
```

```r
## Create Plot

#convert the bearing from a radian for compass bearing
Ohau2$bearing <- Ohau2$Abs.angle*(180/pi)

# choose bin size (degrees/bin)
deg <- 30

# define the range of each bin
dir.breaks <- seq(0-(deg/2), 360+(deg/2), deg)

# generate a factor variable, exchanging the directions with the ranges

# assign each direction to a bin range
dir.binned <- cut(Ohau2$bearing,
                  breaks = dir.breaks,
```

```
                  ordered_result = TRUE)

# generate labels
dir.labels <- as.character(c(seq(0, 360-deg, by = deg), 0))

# replace ranges with bin labels
levels(dir.binned) <- dir.labels

# Assign bin names to the original data set
Ohau2$dir.binned <- dir.binned

# check what values are needed for the ylab
summary(dir.binned)

##   0  30  60  90 120 150 180 210 240 270 300 330
##   4   1   3   4   4   2   3   2   1   5   2   1

# Prep the plot
thm <- theme_bw() +
  theme(axis.text.x = element_text(size=8, face = "plain"),
        axis.text.y = element_text(size=8, face = "plain"),
        #axis.title.x = element_blank(),
        axis.title.y = element_text(face = "plain", hjust = 0.9, vjust =
1.2),
        panel.border = element_blank(),
        panel.grid  = element_blank())

# initialise the plot
plt.dirrose <- ggplot() +
  # add a series of horizontal lines
  geom_hline(yintercept = seq(0, 5, by = 1), colour = "grey60", size = 0.3) +
  # add a darker horizontal line as the top border
  geom_hline(yintercept = 5, colour = "black", size = 0.5) +
  # We want 12 vertical lines representing the centers of the 30° ranges
  geom_vline(xintercept = c(seq(1,12,1)), colour = "grey60", size = 0.3) +
  # draw histogram bars
  geom_bar(data = Ohau2, aes(x = dir.binned), width = 1, colour="black", size
= 0.3, alpha=0.5) +
  # Add the x-axis labels
  scale_x_discrete( drop = FALSE, labels = c(0, "", "", 90, "", "", 180, "",
"", 270, "", "")) +
  # Add the y-axis labels
  scale_y_continuous(limits = c(0, 5), expand = c(0, 0),
                     breaks = c(0, 1, 2, 3, 4, 5),
                     labels = c(0, 1, 2, 3, 4, 5)) +
  # Add the axis titles
  labs(x = 'Bearing (°)', y = 'Count of bearings', title = "Ohau Absolute") +
  #Add an arrow to point out the mean turning angle
  annotate("segment", x = 3.9, xend = 3.9, y = 0, yend = 4.5, colour =
"black", size=3, alpha=0.6, arrow=arrow()) +
```

```
  # wrap the histogram into a windrose
  coord_polar(start = -(deg/2)*(pi/180)) +
  # apply theme
  theme_classic()

# draw the plot
plt.dirrose
```




```
#
# PATERSONS ----
#

#get a list of the radians in a new data set
Pat2 <- data.frame(Abs.angle = as.numeric(Patt_Data$abs.angle))

#get rid of NAs
Pat2 <- na.omit(Pat2)

#convert the negative radians into positive ones
Pat2$Abs.angle[Pat2$Abs.angle<0] <- 2*pi + Pat2$Abs.angle[Pat2$Abs.angle<0]

#Calculate the mean of the cosines and sines:
sum.cos = sum(cos(Pat2$Abs.angle))
sum.sin = sum(sin(Pat2$Abs.angle))

#Take the arctangent:
```

```
x = atan2(sum.sin, sum.cos)

#Convert to degrees:
x.deg = x * (180 / pi)

#Print
x.deg
```

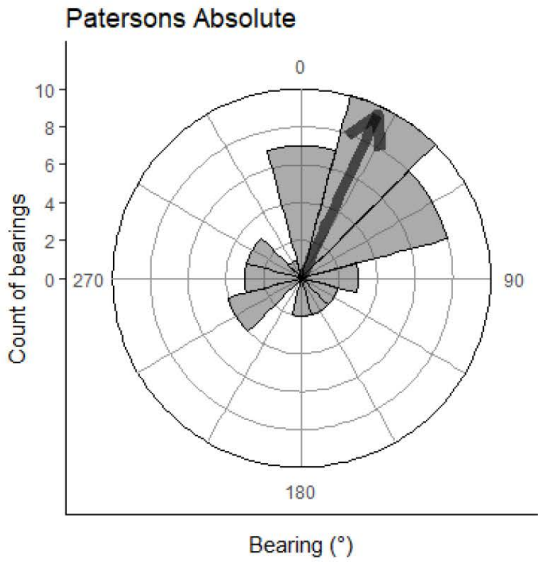
```
## [1] 25.01361
```

```
## Create plot

#convert the bearing from a radian for compass bearing
Pat2$bearing <- Pat2$Abs.angle*(180/pi)

# choose bin size (degrees/bin)
deg <- 30

# define the range of each bin
dir.breaks <- seq(0-(deg/2), 360+(deg/2), deg)

#generate a factor variable, exchanging the directions with the ranges

# assign each direction to a bin range
dir.binned <- cut(Pat2$bearing,
                  breaks = dir.breaks,
                  ordered_result = TRUE)

# generate labels
dir.labels <- as.character(c(seq(0, 360-deg, by = deg), 0))

# replace ranges with bin labels
levels(dir.binned) <- dir.labels

# Assign bin names to the original data set
Pat2$dir.binned <- dir.binned

#check what values are needed for the ylab
summary(dir.binned)
```

```
##   0  30  60  90 120 150 180 210 240 270 300 330
##   7  10   8   3   2   2   2   0   4   3   3   1
```

```
#Prep the plot
thm <- theme_bw() +
  theme(axis.text.x = element_text(size=8, face = "plain"),
        axis.text.y = element_text(size=8, face = "plain"),
        #axis.title.x = element_blank(),
        axis.title.y = element_text(face = "plain", hjust = 0.9, vjust =
1.2),
```

```
        panel.border = element_blank(),
        panel.grid  = element_blank())

# initialise the plot
plt.dirrose <- ggplot() +
  # add a series of horizontal lines
  geom_hline(yintercept = seq(0, 10, by = 2), colour = "grey60", size = 0.3)
+
  # add a darker horizontal line as the top border
  geom_hline(yintercept = 10, colour = "black", size = 0.5) +
  # We want 12 vertical lines representing the centers of the 30° ranges
  geom_vline(xintercept = c(seq(1,12,1)), colour = "grey60", size = 0.3) +
  # draw histogram bars
  geom_bar(data = Pat2, aes(x = dir.binned), width = 1, colour="black", size
= 0.3, alpha=0.5) +
  # Add the x-axis labels
  scale_x_discrete( drop = FALSE, labels = c(0, "", "", 90, "", "", 180, "",
"", 270, "", "")) +
  # Add the y-axis labels
  scale_y_continuous(limits = c(0, 10), expand = c(0, 0),
                     breaks = c(0,  2,  4,  6, 8, 10),
                     labels = c(0,  2,  4,  6,  8,  10)) +
  # Add the axis titles
  labs(x = 'Bearing (°)', y = 'Count of bearings', title = "Patersons
Absolute") +
  #Add an arrow to point out the mean turning angle
  annotate("segment", x = 1.83, xend = 1.83, y = 0, yend = 9.5, colour =
"black", size=3, alpha=0.6, arrow=arrow()) +
  # wrap the histogram into a windrose
  coord_polar(start = -(deg/2)*(pi/180)) +
  # apply theme
  theme_classic()

# draw the plot
plt.dirrose
```




```
# ----------------------
# Analyse turning angles
# ----------------------

library(circular)

##
## Attaching package: 'circular'

## The following objects are masked from 'package:CircStats':
##
##     A1, A1inv, deg, I.0, I.1, I.p, pp.plot, rad, rose.diag,
##     rstable

## The following objects are masked from 'package:stats':
##
##     sd, var

# 1. Compare turning angles using the watson two sample test

# Absolute angles Ohau v. Patersons

#with radians
a <- watson.two.test(Ohau_Data$abs.angle, Patt_Data$abs.angle, alpha=0.05)
a #reject null
```

```
##
##       Watson's Two-Sample Test of Homogeneity
##
## Test Statistic: 0.2533
## Level 0.05 Critical Value: 0.187
## Reject Null Hypothesis
```

```
# Relative angles Ohau v. Patersons

#with radians
b <- watson.two.test(Ohau_Data$rel.angle, Patt_Data$rel.angle, alpha=0.05)
b #do not reject null
```

```
##
##       Watson's Two-Sample Test of Homogeneity
##
## Test Statistic: 0.059
## Level 0.05 Critical Value: 0.187
## Do Not Reject Null Hypothesis
```

```
# 2.The significance of mean tunring angles using the Rayleigh test.

Ohau_AbsRayleigh <- rayleigh.test(Ohau2$Abs.angle)
Ohau_AbsRayleigh
```

```
##
##        Rayleigh Test of Uniformity
##        General Unimodal Alternative
##
## Test Statistic:  0.077
## P-value:  0.8294
```

```
Ohau_RelRayleigh <- rayleigh.test(Ohau1$Rel.angle)
Ohau_RelRayleigh
```

```
##
##        Rayleigh Test of Uniformity
##        General Unimodal Alternative
##
## Test Statistic:  0.0481
## P-value:  0.9515
```

```
Patt_AbsRayleigh <- rayleigh.test(Pat2$Abs.angle)
Patt_AbsRayleigh
```

```
##
##        Rayleigh Test of Uniformity
##        General Unimodal Alternative
##
## Test Statistic:  0.401
## P-value:  6e-04
```

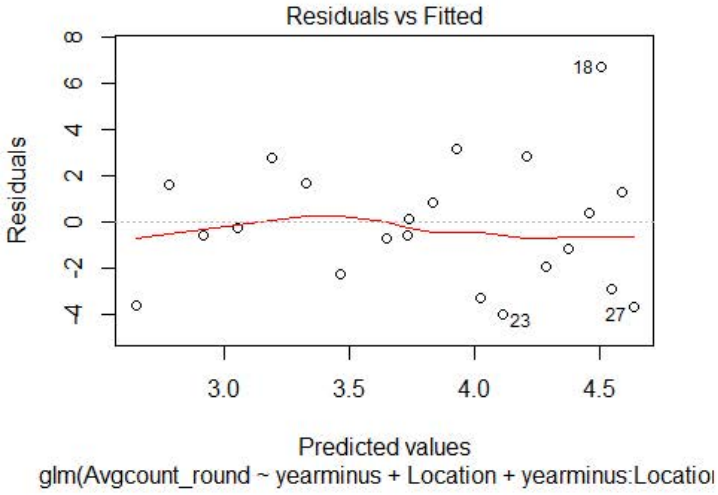
```
Patt_RelRayleigh <- rayleigh.test(Pat1$Rel.angle)
Patt_RelRayleigh

##
##        Rayleigh Test of Uniformity
##        General Unimodal Alternative
##
## Test Statistic:  0.1904
## P-value:  0.2728
```

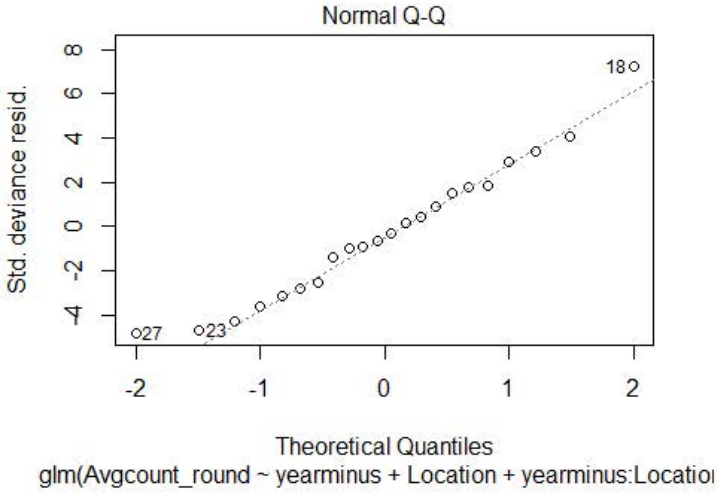
Appendix F

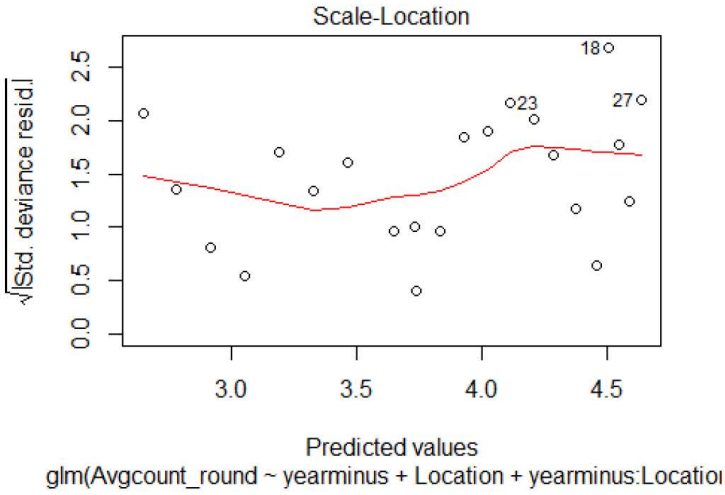
Chapter 4: Statistical analyses and R code

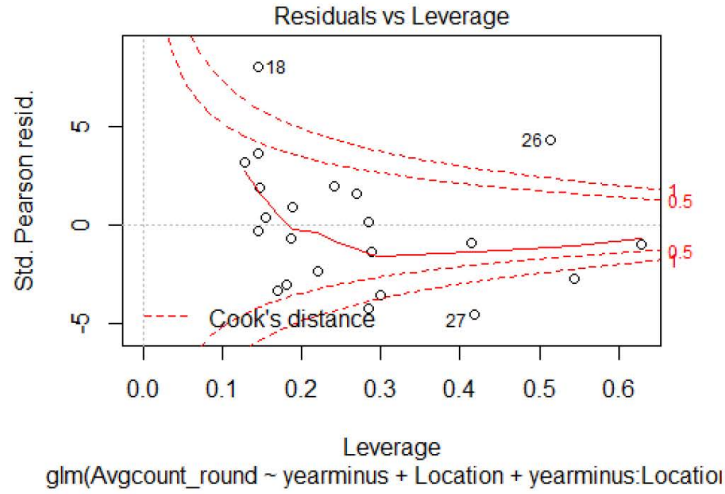
Chapter 4: Evidence that reducing mammalian predators is beneficial for threatened and declining New Zealand grasshoppers

```
# -------------------
# Sigaus minutus data
# -------------------

sig <- read.csv('C:/Users/Jennifer/Desktop/Data chapters/Sigaus/Sigaus max
counts no 2007.csv', header = TRUE)

#rescale x variable.
sig$yearminus <- sig$Year - 2012

library(lme4)

mod1 <- glm(Avgcount_round ~ yearminus, data = sig, family = 'poisson')

mod2 <- glm(Avgcount_round ~ yearminus + Location, data = sig, family =
'poisson')

mod3 <- glm(Avgcount_round ~ Location, data = sig, family = 'poisson')

mod4 <- glm(Avgcount_round ~ yearminus + Location + yearminus:Location, data
= sig, family = 'poisson')

anova(mod1, mod2, mod3, mod4)

## Analysis of Deviance Table
##
## Model 1: Avgcount_round ~ yearminus
## Model 2: Avgcount_round ~ yearminus + Location
## Model 3: Avgcount_round ~ Location
## Model 4: Avgcount_round ~ yearminus + Location + yearminus:Location
##   Resid. Df Resid. Dev Df Deviance
## 1        20     486.90
## 2        18     191.48  2  295.421
## 3        19     194.63 -1   -3.152
## 4        16     150.98  3   43.658

plot(mod4)
```


Residuals vs Fitted
Residuals
Predicted values
glm(Avgcount_round ~ yearminus + Location + yearminus:Locatio
18
23
27


Normal Q-Q
Std. deviance resid.
Theoretical Quantiles
glm(Avgcount_round ~ yearminus + Location + yearminus:Locatio
18
23
27

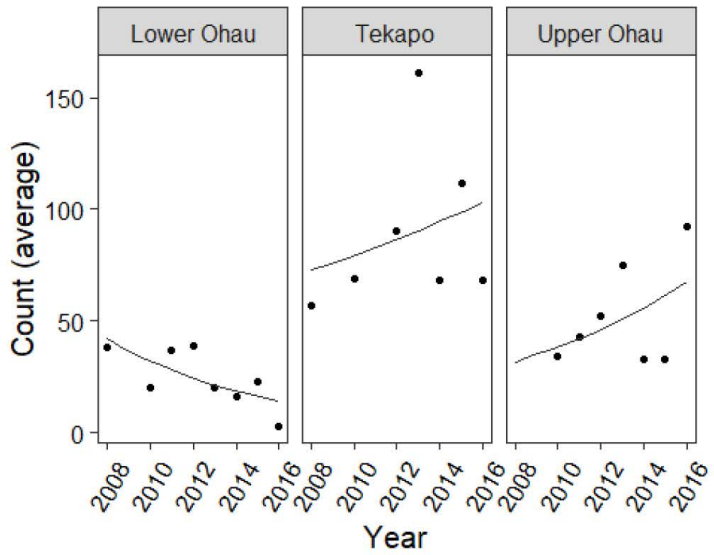





```r
# mod4 has the lowest AIC, and the model fits the data well

summary(mod4)
```

```
##
## Call:
## glm(formula = Avgcount_round ~ yearminus + Location + yearminus:Location,
##     family = "poisson", data = sig)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -3.9673  -2.1749  -0.4343   1.5251   6.6822
##
## Coefficients:
##                              Estimate Std. Error z value Pr(>|z|)
## (Intercept)                   3.19105    0.07276  43.856  < 2e-16 ***
## yearminus                    -0.13598    0.02834  -4.799 1.60e-06 ***
## LocationTekapo                1.26926    0.08414  15.085  < 2e-16 ***
## LocationUpper Ohau            0.64319    0.09689   6.638 3.17e-11 ***
## yearminus:LocationTekapo      0.17987    0.03243   5.546 2.92e-08 ***
## yearminus:LocationUpper Ohau  0.22990    0.03884   5.918 3.25e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for poisson family taken to be 1)
##
##     Null deviance: 492.07  on 21  degrees of freedom
## Residual deviance: 150.98  on 16  degrees of freedom
##   (5 observations deleted due to missingness)
## AIC: 285.55
##
## Number of Fisher Scoring iterations: 4

# ----------------
# Predict new data
# ----------------

newdata <- expand.grid(yearminus=seq(-4,4, length=10),
                       Location=levels(sig$Location))
newdata$locationprediction <- predict(mod4, newdata=newdata)
newdata$populationprediction <- predict(mod4, newdata=newdata, re.form=~0)

# -----------
# Plot model
# -----------

base_size <- 15      #fontsize

ggplot(sig, aes(y=Avgcount_round, x=yearminus+2012)) +
  geom_point() +
  geom_line(data=newdata, aes(y=exp(locationprediction)), colour="grey20") +
  facet_wrap(~Location) +
  theme_bw(base_size = base_size) +
  ylab("Count (average)") +
```

```
  xlab("Year")+
  theme(axis.text.x = element_text(angle = 60, vjust=0.5, size = base_size
*0.8, colour="black"),
        axis.text.y=element_text(size=base_size*0.8, colour="black"),
        panel.grid=element_blank())
```




```
# ------------------------------------------------------------------
# T-tests of the tracking rates of animals in the aviary 2015 - 2016
# ------------------------------------------------------------------

#Hedgehogs
hogmod <- c(50,80,80,80)
hogno <- c(100,100,100,100)

t.test(hogmod,hogno)

##
##  Welch Two Sample t-test
##
## data:  hogmod and hogno
## t = -3.6667, df = 3, p-value = 0.03508
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -51.368347  -3.631653
## sample estimates:
## mean of x mean of y
##      72.5     100.0
```

```r
#Cats
catmod <- c(0,40,60,30)
catno <- c(20,30,60,40)

t.test(catmod, catno)
```

```
##
##  Welch Two Sample t-test
##
## data:  catmod and catno
## t = -0.33029, df = 5.2993, p-value = 0.7538
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -43.26232  33.26232
## sample estimates:
## mean of x mean of y
##      32.5      37.5
```

```r
#Mustelids
mustelidmod <- c(0,10,0,70)
mustelidno <- c(0,0,0,40)

t.test(mustelidmod,mustelidno)
```

```
##
##  Welch Two Sample t-test
##
## data:  mustelidmod and mustelidno
## t = 0.51075, df = 4.8831, p-value = 0.6318
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -40.6938  60.6938
## sample estimates:
## mean of x mean of y
##        20        10
```

```r
#Lizards
lizardmod <- c(50,70,70,60)
lizardno <- c(60,90,30,20)

t.test(lizardmod, lizardno)
```

```
##
##  Welch Two Sample t-test
##
## data:  lizardmod and lizardno
## t = 0.75665, df = 3.5454, p-value = 0.4964
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -35.7825  60.7825
## sample estimates:
```

```
## mean of x mean of y
##      62.5      50.0

#Rodents
rodentmod <- c(20,10,0,0)
rodentno <- c(0,0,10,10)

t.test(rodentmod,rodentno)

##
##  Welch Two Sample t-test
##
## data:  rodentmod and rodentno
## t = 0.44721, df = 4.927, p-value = 0.6737
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -11.93426  16.93426
## sample estimates:
## mean of x mean of y
##       7.5       5.0
```

Appendix G

Chapter 5: Data analyses and R code

Chapter 5: Informing the design of a long-term monitoring protocol for a highly cryptic Nationally Endangered insect: removal sampling as a basis for protocol development

```
# --------------------------------
# compare temperatures in the plot
# --------------------------------

temps <- c(29.3, 26,
25.2,27.2,24.5,27,20.5,32.3,14.4,22.5,21.7,'N/A',26,27.3,27.8,21.8,34.8,21.6,
29.7,14.1,18.6,16.1,20.6,26,28.2,19.7,19.5,25.9,32.5,23,24,26.6,25.5,27,21,28
.6,24.1,18.3,28.3,30.1,23.2,27.3,27.5,21.4,22.4,25.2,25.5,23,12.2,28.4,27.5,2
1.5,30.3,28.9,25.9,33.5,22.6,18,30.6,25.1,24.5,33.6,16.7,28.8,17.2,24.3)

plots <-
c('c','a','a','a','a','a','a','a','a','a','a','b','b','b','b','b','b','b','b'
,'b','b','b','c','c','c','c','c','c','c','c','c','c','c','d','d','d','d','d',
'd','d','d','d','d','d','e','e','e','e','e','e','e','e','e','e','e','f','f','
f','f','f','f','f','f','f','f')

dat <- data.frame(plots, temps)

dat$plots <- as.factor(dat$plots)
dat$temps <- as.numeric(dat$temps)

temp <- aov(temps ~ plots, data = dat)
summary(temp)

##             Df Sum Sq Mean Sq F value Pr(>F)
## plots        5     71   14.29   0.058  0.998
## Residuals   60  14836  247.27

# ----------------------------------------
# compare the vegetation cover in the plots
# ----------------------------------------

library(vegan)

# Load the data
data <- read.csv('C:/Users/Jennifer/Desktop/Data
Chapters/Pattersons/Vegetation2.csv')

#Remove the plots variable
data2 <- data[, 2:8]
```

```r
#Calculate the distance between and among variables
data.dist <- vegdist(data2)

#Conduct ANOSIM analysis among and between plots
data.ano <- anosim(data.dist, data$Plot)

#Output
summary(data.ano)
```

```
##
## Call:
## anosim(x = data.dist, grouping = data$Plot)
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.2098
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
##
## Upper quantiles of permutations (null model):
##    90%    95%  97.5%    99%
## 0.0522 0.0645 0.0809 0.1034
##
## Dissimilarity ranks between and within classes:
##            0%     25%    50%     75%  100%   N
## Between   1.5 122.000 226.00 328.500 434.5 375
## A0      158.5 362.500 380.50 384.500 411.0  10
## A60       8.0 106.875 236.75 270.875 310.5  10
## B0       20.0  86.875 187.75 250.875 286.5  10
## B60       1.5  13.000  43.00 334.000 340.0  10
## C0       23.0  86.125 155.75 166.625 183.5  10
## C60       3.0  46.500  83.50 105.750 138.0  10
```

```r
# Using the adonis test ----

library(devtools)
library(pairwiseAdonis)

## default test by terms
adonis2(data2 ~ Plot, data = data)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = data2 ~ Plot, data = data)
##          Df SumOfSqs      R2      F Pr(>F)
```

```
## Plot      5  0.73172 0.52916 5.3946  0.001 ***
## Residual 24  0.65107 0.47084
## Total    29  1.38279 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

## overall tests
adonis2(data2 ~ Plot, data = data, by = NULL)

## Permutation test for adonis under reduced model
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = data2 ~ Plot, data = data, by = NULL)
##          Df SumOfSqs      R2      F Pr(>F)
## Model     5  0.73172 0.52916 5.3946  0.001 ***
## Residual 24  0.65107 0.47084
## Total    29  1.38279 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

pairwise.adonis(data[,2:8], data$Plot)

##         pairs Df   SumsOfSqs   F.Model         R2 p.value p.adjusted sig
## 1   A0 vs A60  1 0.392787535 5.4957284 0.40721984   0.026      0.390
## 2    A0 vs B0  1 0.369124135 5.2339864 0.39549583   0.022      0.330
## 3   A0 vs B60  1 0.387157416 5.2031501 0.39408399   0.027      0.405
## 4    A0 vs C0  1 0.425325087 6.1901672 0.43622933   0.010      0.150
## 5   A0 vs C60  1 0.497621926 7.2897998 0.47677536   0.006      0.090
## 6   A60 vs B0  1 0.027296200 4.5498596 0.36254267   0.009      0.135
## 7  A60 vs B60  1 0.020676600 2.0921063 0.20730125   0.031      0.465
## 8   A60 vs C0  1 0.005299680 1.2664521 0.13667066   0.187      1.000
## 9  A60 vs C60  1 0.009436188 2.5246335 0.23987852   0.054      0.810
## 10  B0 vs B60  1 0.001590200 0.1779504 0.02175977   0.691      1.000
## 11   B0 vs C0  1 0.014819657 4.5771938 0.36392807   0.021      0.315
## 12  B0 vs C60  1 0.020591117 7.3784870 0.47979278   0.008      0.120
## 13  B60 vs C0  1 0.008044657 1.1296269 0.12373199   0.379      1.000
## 14 B60 vs C60  1 0.012770352 1.9133057 0.19300381   0.024      0.360
## 15  C0 vs C60  1 0.002622708 2.6871643 0.25143847   0.040      0.600

pairwise.adonis(data[,2:8], data$Plot, p.adjust.m='bonferroni')

##         pairs Df   SumsOfSqs   F.Model         R2 p.value p.adjusted sig
## 1   A0 vs A60  1 0.392787535 5.4957284 0.40721984   0.024      0.360
## 2    A0 vs B0  1 0.369124135 5.2339864 0.39549583   0.016      0.240
## 3   A0 vs B60  1 0.387157416 5.2031501 0.39408399   0.021      0.315
## 4    A0 vs C0  1 0.425325087 6.1901672 0.43622933   0.006      0.090
## 5   A0 vs C60  1 0.497621926 7.2897998 0.47677536   0.006      0.090
## 6   A60 vs B0  1 0.027296200 4.5498596 0.36254267   0.009      0.135
## 7  A60 vs B60  1 0.020676600 2.0921063 0.20730125   0.026      0.390
## 8   A60 vs C0  1 0.005299680 1.2664521 0.13667066   0.242      1.000
```

```
## 9  A60 vs C60  1 0.009436188 2.5246335 0.23987852   0.054      0.810
## 10  B0 vs B60  1 0.001590200 0.1779504 0.02175977   0.690      1.000
## 11   B0 vs C0  1 0.014819657 4.5771938 0.36392807   0.026      0.390
## 12  B0 vs C60  1 0.020591117 7.3784870 0.47979278   0.011      0.165
## 13  B60 vs C0  1 0.008044657 1.1296269 0.12373199   0.353      1.000
## 14 B60 vs C60  1 0.012770352 1.9133057 0.19300381   0.022      0.330
## 15  C0 vs C60  1 0.002622708 2.6871643 0.25143847   0.041      0.615

# ---------------------------------------------------------
# compare the mean densities in the plots over the summer
# ---------------------------------------------------------

# Read in the data
dat <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Pattersons/Density
Anova_FIXED.csv')

# anova
dens <- aov(density ~ Plot, data = dat)
summary(dens)

##             Df Sum Sq Mean Sq F value Pr(>F)
## Plot        29 1916.2   66.08   7.657  2e-11 ***
## Residuals   60  517.8    8.63
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

##  post-hoc test

# visual
marginal <- lsmeans(dens, ~ Plot,
                    adjust = "tukey")
(posthoc_dens <- cld(marginal,
                    alpha = 0.05,
                    Letters = letters,
                    adjust = "tukey"))

##  Plot    lsmean   SE df lower.CL upper.CL .group
##  C60_dec  0.772 1.70 60  -4.7989     6.34  a
##  A0_nov   2.315 1.70 60  -3.2557     7.89  ab
##  B60_nov  2.469 1.70 60  -3.1013     8.04  abc
##  B0_nov   2.778 1.70 60  -2.7927     8.35  abc
##  C60_nov  3.395 1.70 60  -2.1754     8.97  abcd
##  B0_dec   3.472 1.47 60  -1.3519     8.30  abc
##  B60_dec  3.549 1.70 60  -2.0211     9.12  abcd
##  A0_dec   3.704 2.08 60  -3.1187    10.53  abcd
##  A60_dec  4.475 1.70 60  -1.0952    10.05  abcd
##  B60_feb  5.093 1.70 60  -0.4779    10.66  abcd
##  B60_jan  5.247 1.70 60  -0.3236    10.82  abcd
##  A0_mar   5.556 1.70 60  -0.0149    11.13  abcd
##  B60_mar  5.864 1.70 60   0.2937    11.43  abcde
```

```
##  C0_dec   6.019 1.70 60   0.4481    11.59  abcde
##  C60_mar  6.327 1.70 60   0.7567    11.90  abcde
##  C60_jan  6.790 1.70 60   1.2197    12.36  abcde
##  A60_mar  6.944 1.70 60   1.3740    12.51  abcde
##  A60_nov  7.716 1.70 60   2.1456    13.29  abcdef
##  B0_mar   7.870 1.70 60   2.2999    13.44  abcdef
##  C0_nov   8.488 1.70 60   2.9172    14.06  abcdef
##  A60_jan  8.951 1.70 60   3.3801    14.52  abcdef
##  B0_feb  10.031 1.70 60   4.4604    15.60  abcdef
##  A0_jan  10.494 1.70 60   4.9234    16.06   bcdef
##  A0_feb  10.648 1.70 60   5.0777    16.22   bcdef
##  B0_jan  11.420 1.70 60   5.8493    16.99   bcdef
##  A60_feb 11.883 1.70 60   6.3122    17.45    cdefg
##  C60_feb 12.809 1.70 60   7.2382    18.38     defg
##  C0_feb  15.278 1.70 60   9.7073    20.85      efg
##  C0_mar  16.821 1.70 60  11.2505    22.39       fg
##  C0_jan  21.142 1.70 60  15.5715    26.71        g
##
## Confidence level used: 0.95
## Conf-level adjustment: sidak method for 30 estimates
## P value adjustment: tukey method for comparing a family of 30 estimates
## significance level used: alpha = 0.05
```

```
# numeric
posthoc <- TukeyHSD(x=dens, 'Plot', conf.level=0.95)
summary(posthoc)
```

```
##      Length Class  Mode
## Plot 1740   -none- numeric
```

```
(kk <- as.data.frame(posthoc$Plot))
```

```
##                         diff          lwr          upr        p adj
## A0_feb-A0_dec     6.94444444  -3.61041073  17.49929961 7.060497e-01
## A0_jan-A0_dec     6.79012346  -3.76473171  17.34497863 7.446112e-01
## A0_mar-A0_dec     1.85185185  -8.70300332  12.40670702 1.000000e+00
## A0_nov-A0_dec    -1.38888889 -11.94374406   9.16596628 1.000000e+00
## A60_dec-A0_dec    0.77160494  -9.78325023  11.32646011 1.000000e+00
## A60_feb-A0_dec    8.17901235  -2.37584282  18.73386752 3.796036e-01
## A60_jan-A0_dec    5.24691358  -5.30794159  15.80176875 9.753915e-01
## A60_mar-A0_dec    3.24074074  -7.31411443  13.79559591 9.999900e-01
## A60_nov-A0_dec    4.01234568  -6.54250949  14.56720085 9.994728e-01
## B0_dec-A0_dec    -0.23148148 -10.24469630   9.78173333 1.000000e+00
## B0_feb-A0_dec     6.32716049  -4.22769468  16.88201567 8.461047e-01
## B0_jan-A0_dec     7.71604938  -2.83880579  18.27090455 4.986566e-01
## B0_mar-A0_dec     4.16666667  -6.38818850  14.72152184 9.990180e-01
## B0_nov-A0_dec    -0.92592593 -11.48078110   9.62892925 1.000000e+00
## B60_dec-A0_dec   -0.15432099 -10.70917616  10.40053418 1.000000e+00
## B60_feb-A0_dec    1.38888889  -9.16596628  11.94374406 1.000000e+00
## B60_jan-A0_dec    1.54320988  -9.01164529  12.09806505 1.000000e+00
## B60_mar-A0_dec    2.16049383  -8.39436134  12.71534900 1.000000e+00
```

```
## B60_nov-A0_dec   -1.23456790 -11.78942307   9.32028727 1.000000e+00
## C0_dec-A0_dec     2.31481481  -8.24004036  12.86966999 1.000000e+00
## C0_feb-A0_dec    11.57407407   1.01921890  22.12892924 1.644238e-02
## C0_jan-A0_dec    17.43827161   6.88341643  27.99312678 6.938080e-06
## C0_mar-A0_dec    13.11728395   2.56242877  23.67213912 2.540324e-03
## C0_nov-A0_dec     4.78395062  -5.77090455  15.33880579 9.923403e-01
## C60_dec-A0_dec   -2.93209877 -13.48695394   7.62275641 9.999988e-01
## C60_feb-A0_dec    9.10493827  -1.44991690  19.65979344 1.929452e-01
## C60_jan-A0_dec    3.08641975  -7.46843542  13.64127493 9.999963e-01
## C60_mar-A0_dec    2.62345679  -7.93139838  13.17831196 9.999999e-01
## C60_nov-A0_dec   -0.30864198 -10.86349715  10.24621320 1.000000e+00
## A0_jan-A0_feb    -0.15432099  -9.59487045   9.28622848 1.000000e+00
## A0_mar-A0_feb    -5.09259259 -14.53314205   4.34795687 9.401619e-01
## A0_nov-A0_feb    -8.33333333 -17.77388279   1.10721613 1.616172e-01
## A60_dec-A0_feb   -6.17283950 -15.61338897   3.26770996 7.169799e-01
## A60_feb-A0_feb    1.23456791  -8.20598156  10.67511737 1.000000e+00
## A60_jan-A0_feb   -1.69753086 -11.13808032   7.74301860 1.000000e+00
## A60_mar-A0_feb   -3.70370370 -13.14425316   5.73684576 9.991120e-01
## A60_nov-A0_feb   -2.93209876 -12.37264823   6.50845070 9.999874e-01
## B0_dec-A0_feb    -7.17592592 -16.00675133   1.65489948 2.896559e-01
## B0_feb-A0_feb    -0.61728395 -10.05783341   8.82326551 1.000000e+00
## B0_jan-A0_feb     0.77160494  -8.66894452  10.21215440 1.000000e+00
## B0_mar-A0_feb    -2.77777778 -12.21832724   6.66277169 9.999958e-01
## B0_nov-A0_feb    -7.87037037 -17.31091983   1.57017909 2.456346e-01
## B60_dec-A0_feb   -7.09876543 -16.53931489   2.34178403 4.404215e-01
## B60_feb-A0_feb   -5.55555555 -14.99610502   3.88499391 8.674179e-01
## B60_jan-A0_feb   -5.40123457 -14.84178403   4.03931490 8.957693e-01
## B60_mar-A0_feb   -4.78395062 -14.22450008   4.65659885 9.693048e-01
## B60_nov-A0_feb   -8.17901234 -17.61956181   1.26153712 1.867932e-01
## C0_dec-A0_feb    -4.62962963 -14.07017909   4.81091983 9.790507e-01
## C0_feb-A0_feb     4.62962963  -4.81091983  14.07017909 9.790507e-01
## C0_jan-A0_feb    10.49382716   1.05327770  19.93437663 1.369967e-02
## C0_mar-A0_feb     6.17283950  -3.26770996  15.61338897 7.169799e-01
## C0_nov-A0_feb    -2.16049383 -11.60104329   7.28005564 1.000000e+00
## C60_dec-A0_feb   -9.87654321 -19.31709267  -0.43599375 2.979488e-02
## C60_feb-A0_feb    2.16049383  -7.28005563  11.60104329 1.000000e+00
## C60_jan-A0_feb   -3.85802469 -13.29857415   5.58252477 9.983013e-01
## C60_mar-A0_feb   -4.32098765 -13.76153711   5.11956181 9.912619e-01
## C60_nov-A0_feb   -7.25308642 -16.69363588   2.18746304 3.967160e-01
## A0_mar-A0_jan    -4.93827161 -14.37882107   4.50227786 9.564852e-01
## A0_nov-A0_jan    -8.17901235 -17.61956181   1.26153712 1.867932e-01
## A60_dec-A0_jan   -6.01851852 -15.45906798   3.42203094 7.593491e-01
## A60_feb-A0_jan    1.38888889  -8.05166057  10.82943835 1.000000e+00
## A60_jan-A0_jan   -1.54320988 -10.98375934   7.89733959 1.000000e+00
## A60_mar-A0_jan   -3.54938272 -12.98993218   5.89116675 9.995625e-01
## A60_nov-A0_jan   -2.77777778 -12.21832724   6.66277168 9.999958e-01
## B0_dec-A0_jan    -7.02160494 -15.85243035   1.80922047 3.296575e-01
## B0_feb-A0_jan    -0.46296296  -9.90351242   8.97758650 1.000000e+00
## B0_jan-A0_jan     0.92592593  -8.51462354  10.36647539 1.000000e+00
## B0_mar-A0_jan    -2.62345679 -12.06400625   6.81709267 9.999987e-01
```

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## B0_nov-A0_jan    -7.71604938 -17.15659885   1.72450008 2.793652e-01
## B60_dec-A0_jan   -6.94444444 -16.38499391   2.49610502 4.857879e-01
## B60_feb-A0_jan   -5.40123457 -14.84178403   4.03931489 8.957693e-01
## B60_jan-A0_jan   -5.24691358 -14.68746304   4.19363588 9.199951e-01
## B60_mar-A0_jan   -4.62962963 -14.07017909   4.81091983 9.790507e-01
## B60_nov-A0_jan   -8.02469136 -17.46524082   1.41585810 2.147783e-01
## C0_dec-A0_jan    -4.47530864 -13.91585810   4.96524082 9.862049e-01
## C0_feb-A0_jan     4.78395062  -4.65659885  14.22450008 9.693048e-01
## C0_jan-A0_jan    10.64814815   1.20759869  20.08869761 1.120022e-02
## C0_mar-A0_jan     6.32716049  -3.11338897  15.76770995 6.723955e-01
## C0_nov-A0_jan    -2.00617284 -11.44672230   7.43437662 1.000000e+00
## C60_dec-A0_jan   -9.72222222 -19.16277168  -0.28167276 3.589975e-02
## C60_feb-A0_jan    2.31481482  -7.12573465  11.75536428 9.999999e-01
## C60_jan-A0_jan   -3.70370370 -13.14425316   5.73684576 9.991120e-01
## C60_mar-A0_jan   -4.16666667 -13.60721613   5.27388280 9.946936e-01
## C60_nov-A0_jan   -7.09876543 -16.53931489   2.34178403 4.404215e-01
## A0_nov-A0_mar    -3.24074074 -12.68129020   6.19980872 9.999131e-01
## A60_dec-A0_mar   -1.08024691 -10.52079638   8.36030255 1.000000e+00
## A60_feb-A0_mar    6.32716050  -3.11338897  15.76770996 6.723955e-01
## A60_jan-A0_mar    3.39506173  -6.04548773  12.83561119 9.997980e-01
## A60_mar-A0_mar    1.38888889  -8.05166057  10.82943835 1.000000e+00
## A60_nov-A0_mar    2.16049383  -7.28005564  11.60104329 1.000000e+00
## B0_dec-A0_mar    -2.08333333 -10.91415874   6.74749207 1.000000e+00
## B0_feb-A0_mar     4.47530864  -4.96524082  13.91585810 9.862049e-01
## B0_jan-A0_mar     5.86419753  -3.57635193  15.30474699 7.988777e-01
## B0_mar-A0_mar     2.31481481  -7.12573465  11.75536428 9.999999e-01
## B0_nov-A0_mar    -2.77777778 -12.21832724   6.66277168 9.999958e-01
## B60_dec-A0_mar   -2.00617284 -11.44672230   7.43437662 1.000000e+00
## B60_feb-A0_mar   -0.46296296  -9.90351242   8.97758650 1.000000e+00
## B60_jan-A0_mar   -0.30864198  -9.74919144   9.13190749 1.000000e+00
## B60_mar-A0_mar    0.30864198  -9.13190749   9.74919144 1.000000e+00
## B60_nov-A0_mar   -3.08641975 -12.52696922   6.35412971 9.999654e-01
## C0_dec-A0_mar     0.46296296  -8.97758650   9.90351243 1.000000e+00
## C0_feb-A0_mar     9.72222222   0.28167276  19.16277168 3.589975e-02
## C0_jan-A0_mar    15.58641975   6.14587029  25.02696922 7.059499e-06
## C0_mar-A0_mar    11.26543209   1.82488263  20.70598156 4.878906e-03
## C0_nov-A0_mar     2.93209876  -6.50845070  12.37264823 9.999874e-01
## C60_dec-A0_mar   -4.78395062 -14.22450008   4.65659884 9.693048e-01
## C60_feb-A0_mar    7.25308642  -2.18746304  16.69363588 3.967160e-01
## C60_jan-A0_mar    1.23456790  -8.20598156  10.67511736 1.000000e+00
## C60_mar-A0_mar    0.77160494  -8.66894452  10.21215440 1.000000e+00
## C60_nov-A0_mar   -2.16049383 -11.60104329   7.28005564 1.000000e+00
## A60_dec-A0_nov    2.16049383  -7.28005563  11.60104329 1.000000e+00
## A60_feb-A0_nov    9.56790124   0.12735177  19.00845070 4.310786e-02
## A60_jan-A0_nov    6.63580247  -2.80474699  16.07635193 5.793483e-01
## A60_mar-A0_nov    4.62962963  -4.81091983  14.07017909 9.790507e-01
## A60_nov-A0_nov    5.40123457  -4.03931489  14.84178403 8.957693e-01
## B0_dec-A0_nov     1.15740741  -7.67341800   9.98823282 1.000000e+00
## B0_feb-A0_nov     7.71604938  -1.72450008  17.15659885 2.793652e-01
## B0_jan-A0_nov     9.10493827  -0.33561119  18.54548773 7.300967e-02
```

```
## B0_mar-A0_nov     5.55555556  -3.88499391  14.99610502 8.674179e-01
## B0_nov-A0_nov     0.46296296  -8.97758650   9.90351243 1.000000e+00
## B60_dec-A0_nov    1.23456790  -8.20598156  10.67511736 1.000000e+00
## B60_feb-A0_nov    2.77777778  -6.66277168  12.21832724 9.999958e-01
## B60_jan-A0_nov    2.93209877  -6.50845070  12.37264823 9.999874e-01
## B60_mar-A0_nov    3.54938272  -5.89116675  12.98993218 9.995625e-01
## B60_nov-A0_nov    0.15432099  -9.28622847   9.59487045 1.000000e+00
## C0_dec-A0_nov     3.70370370  -5.73684576  13.14425317 9.991120e-01
## C0_feb-A0_nov    12.96296296   3.52241350  22.40351242 4.204586e-04
## C0_jan-A0_nov    18.82716049   9.38661103  28.26770996 3.695403e-08
## C0_mar-A0_nov    14.50617283   5.06562337  23.94672230 3.914956e-05
## C0_nov-A0_nov     6.17283951  -3.26770996  15.61338897 7.169799e-01
## C60_dec-A0_nov   -1.54320988 -10.98375934   7.89733959 1.000000e+00
## C60_feb-A0_nov   10.49382716   1.05327770  19.93437662 1.369967e-02
## C60_jan-A0_nov    4.47530864  -4.96524082  13.91585811 9.862049e-01
## C60_mar-A0_nov    4.01234568  -5.42820378  13.45289514 9.969219e-01
## C60_nov-A0_nov    1.08024691  -8.36030255  10.52079638 1.000000e+00
## A60_feb-A60_dec   7.40740741  -2.03314205  16.84795687 3.550990e-01
## A60_jan-A60_dec   4.47530864  -4.96524082  13.91585810 9.862049e-01
## A60_mar-A60_dec   2.46913580  -6.97141366  11.90968526 9.999997e-01
## A60_nov-A60_dec   3.24074074  -6.19980872  12.68129020 9.999131e-01
## B0_dec-A60_dec   -1.00308642  -9.83391183   7.82773899 1.000000e+00
## B0_feb-A60_dec    5.55555556  -3.88499391  14.99610502 8.674179e-01
## B0_jan-A60_dec    6.94444444  -2.49610502  16.38499391 4.857879e-01
## B0_mar-A60_dec    3.39506173  -6.04548773  12.83561119 9.997980e-01
## B0_nov-A60_dec   -1.69753086 -11.13808033   7.74301860 1.000000e+00
## B60_dec-A60_dec  -0.92592593 -10.36647539   8.51462354 1.000000e+00
## B60_feb-A60_dec   0.61728395  -8.82326551  10.05783341 1.000000e+00
## B60_jan-A60_dec   0.77160494  -8.66894452  10.21215440 1.000000e+00
## B60_mar-A60_dec   1.38888889  -8.05166057  10.82943835 1.000000e+00
## B60_nov-A60_dec  -2.00617284 -11.44672230   7.43437662 1.000000e+00
## C0_dec-A60_dec    1.54320988  -7.89733959  10.98375934 1.000000e+00
## C0_feb-A60_dec   10.80246913   1.36191967  20.24301860 9.132682e-03
## C0_jan-A60_dec   16.66666667   7.22611721  26.10721613 1.240348e-06
## C0_mar-A60_dec   12.34567901   2.90512955  21.78622847 1.049992e-03
## C0_nov-A60_dec    4.01234568  -5.42820378  13.45289514 9.969219e-01
## C60_dec-A60_dec  -3.70370370 -13.14425317   5.73684576 9.991120e-01
## C60_feb-A60_dec   8.33333333  -1.10721613  17.77388280 1.616172e-01
## C60_jan-A60_dec   2.31481482  -7.12573465  11.75536428 9.999999e-01
## C60_mar-A60_dec   1.85185185  -7.58869761  11.29240131 1.000000e+00
## C60_nov-A60_dec  -1.08024691 -10.52079638   8.36030255 1.000000e+00
## A60_jan-A60_feb  -2.93209877 -12.37264823   6.50845069 9.999874e-01
## A60_mar-A60_feb  -4.93827161 -14.37882107   4.50227785 9.564852e-01
## A60_nov-A60_feb  -4.16666667 -13.60721613   5.27388279 9.946936e-01
## B0_dec-A60_feb   -8.41049383 -17.24131924   0.42033158 8.265123e-02
## B0_feb-A60_feb   -1.85185185 -11.29240132   7.58869761 1.000000e+00
## B0_jan-A60_feb   -0.46296296  -9.90351243   8.97758650 1.000000e+00
## B0_mar-A60_feb   -4.01234568 -13.45289514   5.42820378 9.969219e-01
## B0_nov-A60_feb   -9.10493827 -18.54548774   0.33561119 7.300966e-02
## B60_dec-A60_feb  -8.33333334 -17.77388280   1.10721613 1.616172e-01
```

```
## B60_feb-A60_feb  -6.79012346 -16.23067292   2.65042600 5.322964e-01
## B60_jan-A60_feb  -6.63580247 -16.07635193   2.80474699 5.793483e-01
## B60_mar-A60_feb  -6.01851852 -15.45906798   3.42203094 7.593491e-01
## B60_nov-A60_feb  -9.41358025 -18.85412971   0.02696921 5.157887e-02
## C0_dec-A60_feb   -5.86419753 -15.30474700   3.57635193 7.988777e-01
## C0_feb-A60_feb    3.39506172  -6.04548774  12.83561119 9.997980e-01
## C0_jan-A60_feb    9.25925926  -0.18129020  18.69980872 6.148509e-02
## C0_mar-A60_feb    4.93827160  -4.50227786  14.37882106 9.564852e-01
## C0_nov-A60_feb   -3.39506173 -12.83561119   6.04548773 9.997980e-01
## C60_dec-A60_feb -11.11111111 -20.55166058  -1.67056165 6.026987e-03
## C60_feb-A60_feb   0.92592592  -8.51462354  10.36647539 1.000000e+00
## C60_jan-A60_feb  -5.09259259 -14.53314206   4.34795687 9.401619e-01
## C60_mar-A60_feb  -5.55555556 -14.99610502   3.88499390 8.674179e-01
## C60_nov-A60_feb  -8.48765432 -17.92820379   0.95289514 1.391409e-01
## A60_mar-A60_jan  -2.00617284 -11.44672230   7.43437662 1.000000e+00
## A60_nov-A60_jan  -1.23456790 -10.67511736   8.20598156 1.000000e+00
## B0_dec-A60_jan   -5.47839506 -14.30922047   3.35243035 8.007282e-01
## B0_feb-A60_jan    1.08024691  -8.36030255  10.52079638 1.000000e+00
## B0_jan-A60_jan    2.46913580  -6.97141366  11.90968527 9.999997e-01
## B0_mar-A60_jan   -1.08024691 -10.52079638   8.36030255 1.000000e+00
## B0_nov-A60_jan   -6.17283951 -15.61338897   3.26770996 7.169799e-01
## B60_dec-A60_jan  -5.40123457 -14.84178403   4.03931489 8.957693e-01
## B60_feb-A60_jan  -3.85802469 -13.29857415   5.58252477 9.983013e-01
## B60_jan-A60_jan  -3.70370370 -13.14425317   5.73684576 9.991120e-01
## B60_mar-A60_jan  -3.08641975 -12.52696922   6.35412971 9.999654e-01
## B60_nov-A60_jan  -6.48148148 -15.92203094   2.95906798 6.262816e-01
## C0_dec-A60_jan   -2.93209877 -12.37264823   6.50845070 9.999874e-01
## C0_feb-A60_jan    6.32716049  -3.11338897  15.76770995 6.723955e-01
## C0_jan-A60_jan   12.19135803   2.75080856  21.63190749 1.314841e-03
## C0_mar-A60_jan    7.87037037  -1.57017910  17.31091983 2.456346e-01
## C0_nov-A60_jan   -0.46296296  -9.90351243   8.97758650 1.000000e+00
## C60_dec-A60_jan  -8.17901235 -17.61956181   1.26153712 1.867932e-01
## C60_feb-A60_jan   3.85802469  -5.58252477  13.29857415 9.983013e-01
## C60_jan-A60_jan  -2.16049383 -11.60104329   7.28005564 1.000000e+00
## C60_mar-A60_jan  -2.62345679 -12.06400625   6.81709267 9.999987e-01
## C60_nov-A60_jan  -5.55555556 -14.99610502   3.88499391 8.674179e-01
## A60_nov-A60_mar   0.77160494  -8.66894452  10.21215440 1.000000e+00
## B0_dec-A60_mar   -3.47222222 -12.30304763   5.35860319 9.990793e-01
## B0_feb-A60_mar    3.08641975  -6.35412971  12.52696922 9.999654e-01
## B0_jan-A60_mar    4.47530864  -4.96524082  13.91585811 9.862049e-01
## B0_mar-A60_mar    0.92592593  -8.51462354  10.36647539 1.000000e+00
## B0_nov-A60_mar   -4.16666667 -13.60721613   5.27388280 9.946936e-01
## B60_dec-A60_mar  -3.39506173 -12.83561119   6.04548773 9.997980e-01
## B60_feb-A60_mar  -1.85185185 -11.29240131   7.58869761 1.000000e+00
## B60_jan-A60_mar  -1.69753086 -11.13808033   7.74301860 1.000000e+00
## B60_mar-A60_mar  -1.08024691 -10.52079638   8.36030255 1.000000e+00
## B60_nov-A60_mar  -4.47530864 -13.91585810   4.96524082 9.862049e-01
## C0_dec-A60_mar   -0.92592593 -10.36647539   8.51462354 1.000000e+00
## C0_feb-A60_mar    8.33333333  -1.10721613  17.77388279 1.616172e-01
## C0_jan-A60_mar   14.19753087   4.75698140  23.63808033 6.344670e-05
```

```
## C0_mar-A60_mar    9.87654321   0.43599374  19.31709267 2.979488e-02
## C0_nov-A60_mar    1.54320988  -7.89733959  10.98375934 1.000000e+00
## C60_dec-A60_mar  -6.17283951 -15.61338897   3.26770996 7.169799e-01
## C60_feb-A60_mar   5.86419753  -3.57635193  15.30474699 7.988777e-01
## C60_jan-A60_mar  -0.15432099  -9.59487045   9.28622848 1.000000e+00
## C60_mar-A60_mar  -0.61728395 -10.05783341   8.82326551 1.000000e+00
## C60_nov-A60_mar  -3.54938272 -12.98993218   5.89116675 9.995625e-01
## B0_dec-A60_nov   -4.24382716 -13.07465257   4.58699825 9.836311e-01
## B0_feb-A60_nov    2.31481482  -7.12573465  11.75536428 9.999999e-01
## B0_jan-A60_nov    3.70370370  -5.73684576  13.14425317 9.991120e-01
## B0_mar-A60_nov    0.15432099  -9.28622847   9.59487045 1.000000e+00
## B0_nov-A60_nov   -4.93827160 -14.37882107   4.50227786 9.564852e-01
## B60_dec-A60_nov  -4.16666667 -13.60721613   5.27388280 9.946936e-01
## B60_feb-A60_nov  -2.62345679 -12.06400625   6.81709267 9.999987e-01
## B60_jan-A60_nov  -2.46913580 -11.90968526   6.97141366 9.999997e-01
## B60_mar-A60_nov  -1.85185185 -11.29240131   7.58869761 1.000000e+00
## B60_nov-A60_nov  -5.24691358 -14.68746304   4.19363588 9.199951e-01
## C0_dec-A60_nov   -1.69753086 -11.13808033   7.74301860 1.000000e+00
## C0_feb-A60_nov    7.56172839  -1.87882107  17.00227786 3.159028e-01
## C0_jan-A60_nov   13.42592593   3.98537647  22.86647539 2.086402e-04
## C0_mar-A60_nov    9.10493827  -0.33561119  18.54548773 7.300967e-02
## C0_nov-A60_nov    0.77160494  -8.66894452  10.21215440 1.000000e+00
## C60_dec-A60_nov  -6.94444444 -16.38499391   2.49610502 4.857879e-01
## C60_feb-A60_nov   5.09259259  -4.34795687  14.53314206 9.401619e-01
## C60_jan-A60_nov  -0.92592592 -10.36647539   8.51462354 1.000000e+00
## C60_mar-A60_nov  -1.38888889 -10.82943835   8.05166057 1.000000e+00
## C60_nov-A60_nov  -4.32098765 -13.76153712   5.11956181 9.912619e-01
## B0_feb-B0_dec     6.55864198  -2.27218343  15.38946738 4.659075e-01
## B0_jan-B0_dec     7.94753086  -0.88329454  16.77835627 1.379631e-01
## B0_mar-B0_dec     4.39814815  -4.43267726  13.22897356 9.748444e-01
## B0_nov-B0_dec    -0.69444444  -9.52526985   8.13638096 1.000000e+00
## B60_dec-B0_dec    0.07716049  -8.75366491   8.90798590 1.000000e+00
## B60_feb-B0_dec    1.62037037  -7.21045504  10.45119578 1.000000e+00
## B60_jan-B0_dec    1.77469136  -7.05613405  10.60551677 1.000000e+00
## B60_mar-B0_dec    2.39197531  -6.43885010  11.22280072 9.999993e-01
## B60_nov-B0_dec   -1.00308642  -9.83391183   7.82773899 1.000000e+00
## C0_dec-B0_dec     2.54629630  -6.28452911  11.37712170 9.999973e-01
## C0_feb-B0_dec    11.80555555   2.97473015  20.63638096 7.004748e-04
## C0_jan-B0_dec    17.66975309   8.83892768  26.50057850 3.337175e-08
## C0_mar-B0_dec    13.34876543   4.51794002  22.17959084 5.662627e-05
## C0_nov-B0_dec     5.01543210  -3.81539331  13.84625751 9.023651e-01
## C60_dec-B0_dec   -2.70061728 -11.53144269   6.13020812 9.999907e-01
## C60_feb-B0_dec    9.33641975   0.50559435  18.16724516 2.621256e-02
## C60_jan-B0_dec    3.31790124  -5.51292417  12.14872664 9.995675e-01
## C60_mar-B0_dec    2.85493827  -5.97588714  11.68576368 9.999722e-01
## C60_nov-B0_dec   -0.07716049  -8.90798590   8.75366491 1.000000e+00
## B0_jan-B0_feb     1.38888889  -8.05166057  10.82943835 1.000000e+00
## B0_mar-B0_feb    -2.16049383 -11.60104329   7.28005563 1.000000e+00
## B0_nov-B0_feb    -7.25308642 -16.69363588   2.18746304 3.967160e-01
## B60_dec-B0_feb   -6.48148148 -15.92203094   2.95906798 6.262816e-01
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## B60_feb-B0_feb   -4.93827161 -14.37882107   4.50227786 9.564852e-01
## B60_jan-B0_feb   -4.78395062 -14.22450008   4.65659884 9.693048e-01
## B60_mar-B0_feb   -4.16666667 -13.60721613   5.27388279 9.946936e-01
## B60_nov-B0_feb   -7.56172840 -17.00227786   1.87882107 3.159028e-01
## C0_dec-B0_feb    -4.01234568 -13.45289514   5.42820378 9.969219e-01
## C0_feb-B0_feb     5.24691358  -4.19363588  14.68746304 9.199951e-01
## C0_jan-B0_feb    11.11111111   1.67056165  20.55166057 6.026987e-03
## C0_mar-B0_feb     6.79012345  -2.65042601  16.23067291 5.322965e-01
## C0_nov-B0_feb    -1.54320988 -10.98375934   7.89733958 1.000000e+00
## C60_dec-B0_feb   -9.25925926 -18.69980872   0.18129020 6.148509e-02
## C60_feb-B0_feb    2.77777778  -6.66277168  12.21832724 9.999958e-01
## C60_jan-B0_feb   -3.24074074 -12.68129020   6.19980872 9.999131e-01
## C60_mar-B0_feb   -3.70370370 -13.14425317   5.73684576 9.991120e-01
## C60_nov-B0_feb   -6.63580247 -16.07635193   2.80474699 5.793483e-01
## B0_mar-B0_jan    -3.54938272 -12.98993218   5.89116675 9.995625e-01
## B0_nov-B0_jan    -8.64197531 -18.08252477   0.79857415 1.192193e-01
## B60_dec-B0_jan   -7.87037037 -17.31091983   1.57017909 2.456346e-01
## B60_feb-B0_jan   -6.32716049 -15.76770996   3.11338897 6.723955e-01
## B60_jan-B0_jan   -6.17283951 -15.61338897   3.26770996 7.169799e-01
## B60_mar-B0_jan   -5.55555556 -14.99610502   3.88499391 8.674179e-01
## B60_nov-B0_jan   -8.95061728 -18.39116675   0.48993218 8.634369e-02
## C0_dec-B0_jan    -5.40123457 -14.84178403   4.03931489 8.957693e-01
## C0_feb-B0_jan     3.85802469  -5.58252477  13.29857415 9.983013e-01
## C0_jan-B0_jan     9.72222222   0.28167276  19.16277169 3.589975e-02
## C0_mar-B0_jan     5.40123456  -4.03931490  14.84178403 8.957693e-01
## C0_nov-B0_jan    -2.93209877 -12.37264823   6.50845070 9.999874e-01
## C60_dec-B0_jan  -10.64814815 -20.08869761  -1.20759869 1.120022e-02
## C60_feb-B0_jan    1.38888889  -8.05166057  10.82943835 1.000000e+00
## C60_jan-B0_jan   -4.62962963 -14.07017909   4.81091983 9.790507e-01
## C60_mar-B0_jan   -5.09259259 -14.53314206   4.34795687 9.401619e-01
## C60_nov-B0_jan   -8.02469136 -17.46524082   1.41585810 2.147783e-01
## B0_nov-B0_mar    -5.09259259 -14.53314205   4.34795687 9.401619e-01
## B60_dec-B0_mar   -4.32098765 -13.76153712   5.11956181 9.912619e-01
## B60_feb-B0_mar   -2.77777778 -12.21832724   6.66277168 9.999958e-01
## B60_jan-B0_mar   -2.62345679 -12.06400625   6.81709267 9.999987e-01
## B60_mar-B0_mar   -2.00617284 -11.44672230   7.43437662 1.000000e+00
## B60_nov-B0_mar   -5.40123457 -14.84178403   4.03931489 8.957693e-01
## C0_dec-B0_mar    -1.85185185 -11.29240131   7.58869761 1.000000e+00
## C0_feb-B0_mar     7.40740741  -2.03314206  16.84795687 3.550990e-01
## C0_jan-B0_mar    13.27160494   3.83105548  22.71215440 2.638627e-04
## C0_mar-B0_mar     8.95061728  -0.48993218  18.39116674 8.634369e-02
## C0_nov-B0_mar     0.61728395  -8.82326551  10.05783341 1.000000e+00
## C60_dec-B0_mar   -7.09876543 -16.53931489   2.34178403 4.404215e-01
## C60_feb-B0_mar    4.93827161  -4.50227786  14.37882107 9.564852e-01
## C60_jan-B0_mar   -1.08024691 -10.52079637   8.36030255 1.000000e+00
## C60_mar-B0_mar   -1.54320988 -10.98375934   7.89733959 1.000000e+00
## C60_nov-B0_mar   -4.47530864 -13.91585810   4.96524082 9.862049e-01
## B60_dec-B0_nov    0.77160494  -8.66894452  10.21215440 1.000000e+00
## B60_feb-B0_nov    2.31481481  -7.12573465  11.75536428 9.999999e-01
## B60_jan-B0_nov    2.46913580  -6.97141366  11.90968526 9.999997e-01
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## B60_mar-B0_nov    3.08641975  -6.35412971  12.52696922 9.999654e-01
## B60_nov-B0_nov   -0.30864198  -9.74919144   9.13190749 1.000000e+00
## C0_dec-B0_nov     3.24074074  -6.19980872  12.68129020 9.999131e-01
## C0_feb-B0_nov    12.50000000   3.05945054  21.94054946 8.371363e-04
## C0_jan-B0_nov    18.36419753   8.92364807  27.80474699 7.856653e-08
## C0_mar-B0_nov    14.04320987   4.60266041  23.48375933 8.066308e-05
## C0_nov-B0_nov     5.70987654  -3.73067292  15.15042600 8.350354e-01
## C60_dec-B0_nov   -2.00617284 -11.44672230   7.43437662 1.000000e+00
## C60_feb-B0_nov   10.03086420   0.59031474  19.47141366 2.464732e-02
## C60_jan-B0_nov    4.01234568  -5.42820378  13.45289514 9.969219e-01
## C60_mar-B0_nov    3.54938272  -5.89116675  12.98993218 9.995625e-01
## C60_nov-B0_nov    0.61728395  -8.82326551  10.05783341 1.000000e+00
## B60_feb-B60_dec   1.54320988  -7.89733959  10.98375934 1.000000e+00
## B60_jan-B60_dec   1.69753086  -7.74301860  11.13808033 1.000000e+00
## B60_mar-B60_dec   2.31481481  -7.12573465  11.75536428 9.999999e-01
## B60_nov-B60_dec  -1.08024691 -10.52079638   8.36030255 1.000000e+00
## C0_dec-B60_dec    2.46913580  -6.97141366  11.90968526 9.999997e-01
## C0_feb-B60_dec   11.72839506   2.28784560  21.16894452 2.554983e-03
## C0_jan-B60_dec   17.59259259   8.15204313  27.03314206 2.759931e-07
## C0_mar-B60_dec   13.27160493   3.83105547  22.71215440 2.638627e-04
## C0_nov-B60_dec    4.93827160  -4.50227786  14.37882107 9.564852e-01
## C60_dec-B60_dec  -2.77777778 -12.21832724   6.66277168 9.999958e-01
## C60_feb-B60_dec   9.25925926  -0.18129020  18.69980872 6.148509e-02
## C60_jan-B60_dec   3.24074074  -6.19980872  12.68129020 9.999131e-01
## C60_mar-B60_dec   2.77777778  -6.66277168  12.21832724 9.999958e-01
## C60_nov-B60_dec  -0.15432099  -9.59487045   9.28622847 1.000000e+00
## B60_jan-B60_feb   0.15432099  -9.28622847   9.59487045 1.000000e+00
## B60_mar-B60_feb   0.77160494  -8.66894452  10.21215440 1.000000e+00
## B60_nov-B60_feb  -2.62345679 -12.06400625   6.81709267 9.999987e-01
## C0_dec-B60_feb    0.92592593  -8.51462354  10.36647539 1.000000e+00
## C0_feb-B60_feb   10.18518518   0.74463572  19.62573465 2.032525e-02
## C0_jan-B60_feb   16.04938272   6.60883325  25.48993218 3.358773e-06
## C0_mar-B60_feb   11.72839506   2.28784559  21.16894452 2.554983e-03
## C0_nov-B60_feb    3.39506173  -6.04548773  12.83561119 9.997980e-01
## C60_dec-B60_feb  -4.32098765 -13.76153712   5.11956181 9.912619e-01
## C60_feb-B60_feb   7.71604938  -1.72450008  17.15659885 2.793652e-01
## C60_jan-B60_feb   1.69753087  -7.74301860  11.13808033 1.000000e+00
## C60_mar-B60_feb   1.23456790  -8.20598156  10.67511736 1.000000e+00
## C60_nov-B60_feb  -1.69753086 -11.13808033   7.74301860 1.000000e+00
## B60_mar-B60_jan   0.61728395  -8.82326551  10.05783341 1.000000e+00
## B60_nov-B60_jan  -2.77777778 -12.21832724   6.66277168 9.999958e-01
## C0_dec-B60_jan    0.77160494  -8.66894452  10.21215440 1.000000e+00
## C0_feb-B60_jan   10.03086420   0.59031473  19.47141366 2.464732e-02
## C0_jan-B60_jan   15.89506173   6.45451227  25.33561119 4.304421e-06
## C0_mar-B60_jan   11.57407407   2.13352461  21.01462353 3.176330e-03
## C0_nov-B60_jan    3.24074074  -6.19980872  12.68129020 9.999131e-01
## C60_dec-B60_jan  -4.47530864 -13.91585810   4.96524082 9.862049e-01
## C60_feb-B60_jan   7.56172840  -1.87882107  17.00227786 3.159028e-01
## C60_jan-B60_jan   1.54320988  -7.89733958  10.98375934 1.000000e+00
## C60_mar-B60_jan   1.08024691  -8.36030255  10.52079638 1.000000e+00
```

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## C60_nov-B60_jan  -1.85185185 -11.29240131   7.58869761 1.000000e+00
## B60_nov-B60_mar  -3.39506173 -12.83561119   6.04548773 9.997980e-01
## C0_dec-B60_mar    0.15432099  -9.28622847   9.59487045 1.000000e+00
## C0_feb-B60_mar    9.41358025  -0.02696922  18.85412971 5.157887e-02
## C0_jan-B60_mar   15.27777778   5.83722832  24.71832724 1.155367e-05
## C0_mar-B60_mar   10.95679012   1.51624066  20.39733958 7.428034e-03
## C0_nov-B60_mar    2.62345679  -6.81709267  12.06400625 9.999987e-01
## C60_dec-B60_mar  -5.09259259 -14.53314205   4.34795687 9.401619e-01
## C60_feb-B60_mar   6.94444445  -2.49610502  16.38499391 4.857879e-01
## C60_jan-B60_mar   0.92592593  -8.51462353  10.36647539 1.000000e+00
## C60_mar-B60_mar   0.46296296  -8.97758650   9.90351243 1.000000e+00
## C60_nov-B60_mar  -2.46913580 -11.90968526   6.97141366 9.999997e-01
## C0_dec-B60_nov    3.54938272  -5.89116675  12.98993218 9.995625e-01
## C0_feb-B60_nov   12.80864197   3.36809251  22.24919144 5.297062e-04
## C0_jan-B60_nov   18.67283951   9.23229005  28.11338897 4.751641e-08
## C0_mar-B60_nov   14.35185185   4.91130239  23.79240131 4.986007e-05
## C0_nov-B60_nov    6.01851852  -3.42203094  15.45906798 7.593491e-01
## C60_dec-B60_nov  -1.69753086 -11.13808033   7.74301860 1.000000e+00
## C60_feb-B60_nov  10.33950617   0.89895671  19.78005564 1.671081e-02
## C60_jan-B60_nov   4.32098766  -5.11956181  13.76153712 9.912619e-01
## C60_mar-B60_nov   3.85802469  -5.58252477  13.29857415 9.983013e-01
## C60_nov-B60_nov   0.92592593  -8.51462354  10.36647539 1.000000e+00
## C0_feb-C0_dec     9.25925926  -0.18129020  18.69980872 6.148509e-02
## C0_jan-C0_dec    15.12345679   5.68290733  24.56400625 1.476762e-05
## C0_mar-C0_dec    10.80246913   1.36191967  20.24301859 9.132682e-03
## C0_nov-C0_dec     2.46913580  -6.97141366  11.90968526 9.999997e-01
## C60_dec-C0_dec   -5.24691358 -14.68746304   4.19363588 9.199951e-01
## C60_feb-C0_dec    6.79012346  -2.65042600  16.23067292 5.322965e-01
## C60_jan-C0_dec    0.77160494  -8.66894452  10.21215440 1.000000e+00
## C60_mar-C0_dec    0.30864198  -9.13190749   9.74919144 1.000000e+00
## C60_nov-C0_dec   -2.62345679 -12.06400625   6.81709267 9.999987e-01
## C0_jan-C0_feb     5.86419753  -3.57635193  15.30474700 7.988777e-01
## C0_mar-C0_feb     1.54320987  -7.89733959  10.98375934 1.000000e+00
## C0_nov-C0_feb    -6.79012346 -16.23067292   2.65042601 5.322965e-01
## C60_dec-C0_feb  -14.50617284 -23.94672230  -5.06562338 3.914956e-05
## C60_feb-C0_feb   -2.46913580 -11.90968526   6.97141366 9.999997e-01
## C60_jan-C0_feb   -8.48765432 -17.92820378   0.95289514 1.391409e-01
## C60_mar-C0_feb   -8.95061728 -18.39116674   0.48993218 8.634369e-02
## C60_nov-C0_feb  -11.88271605 -21.32326551  -2.44216659 2.051205e-03
## C0_mar-C0_jan    -4.32098766 -13.76153712   5.11956180 9.912619e-01
## C0_nov-C0_jan   -12.65432099 -22.09487045  -3.21377153 6.664012e-04
## C60_dec-C0_jan  -20.37037037 -29.81091983 -10.92982091 3.018560e-09
## C60_feb-C0_jan   -8.33333333 -17.77388280   1.10721613 1.616172e-01
## C60_jan-C0_jan  -14.35185185 -23.79240131  -4.91130239 4.986007e-05
## C60_mar-C0_jan  -14.81481482 -24.25536428  -5.37426535 2.407929e-05
## C60_nov-C0_jan  -17.74691358 -27.18746304  -8.30636412 2.147066e-07
## C0_nov-C0_mar    -8.33333333 -17.77388279   1.10721613 1.616172e-01
## C60_dec-C0_mar  -16.04938271 -25.48993217  -6.60883325 3.358773e-06
## C60_feb-C0_mar   -4.01234567 -13.45289514   5.42820379 9.969219e-01
## C60_jan-C0_mar  -10.03086419 -19.47141365  -0.59031473 2.464732e-02
```
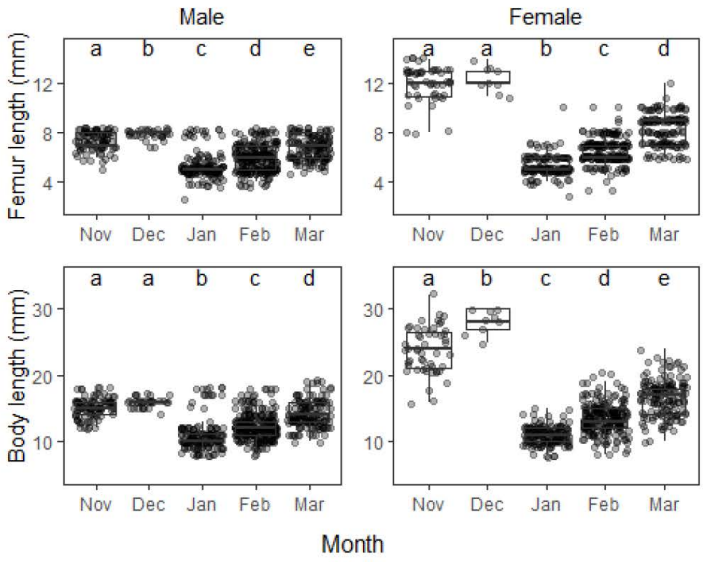

```
## C60_mar-C0_mar  -10.49382716 -19.93437662  -1.05327769 1.369967e-02
## C60_nov-C0_mar  -13.42592592 -22.86647538  -3.98537646 2.086402e-04
## C60_dec-C0_nov   -7.71604938 -17.15659884   1.72450008 2.793652e-01
## C60_feb-C0_nov    4.32098766  -5.11956181  13.76153712 9.912619e-01
## C60_jan-C0_nov   -1.69753086 -11.13808032   7.74301860 1.000000e+00
## C60_mar-C0_nov   -2.16049383 -11.60104329   7.28005564 1.000000e+00
## C60_nov-C0_nov   -5.09259259 -14.53314205   4.34795687 9.401619e-01
## C60_feb-C60_dec  12.03703704   2.59648758  21.47758650 1.643712e-03
## C60_jan-C60_dec   6.01851852  -3.42203094  15.45906798 7.593491e-01
## C60_mar-C60_dec   5.55555556  -3.88499391  14.99610502 8.674179e-01
## C60_nov-C60_dec   2.62345679  -6.81709267  12.06400625 9.999987e-01
## C60_jan-C60_feb  -6.01851852 -15.45906798   3.42203094 7.593491e-01
## C60_mar-C60_feb  -6.48148148 -15.92203094   2.95906798 6.262816e-01
## C60_nov-C60_feb  -9.41358025 -18.85412971   0.02696921 5.157887e-02
## C60_mar-C60_jan  -0.46296296  -9.90351243   8.97758650 1.000000e+00
## C60_nov-C60_jan  -3.39506173 -12.83561119   6.04548773 9.997980e-01
## C60_nov-C60_mar  -2.93209877 -12.37264823   6.50845070 9.999874e-01

# ----------------------------------------
# correlation between femur and body length
# ----------------------------------------

dat <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Pattersons/Full
removal study dataset_FIXED.csv')

library(ggplot2)

# --------------
# juvenile males
# --------------

jm <- dat[dat$Identity == 'mj', ]

jm$Femur <- as.numeric(levels(jm$Femur))[jm$Femur]
jm$Length <- as.numeric(levels(jm$Length))[jm$Length]

# juvenile male femur length
mfem <- ggplot(data = jm, mapping = aes(x = Month, y = Femur)) +
  geom_jitter(alpha = 0.3) +
  geom_boxplot(alpha = 0) +
  scale_x_discrete(limits=c("Nov", "Dec", "Jan", "Feb", "Mar")) +
  theme_bw () +
  theme(panel.grid = element_blank(),
        axis.title.x=element_blank(),
        plot.title = element_text(size = rel(1.05), hjust = 0.5,
        margin = margin(t = 5, b = 5, unit = "pt"))) +
  #theme(text = element_text(size=20)) +
  ylab("Femur length (mm)") +
    labs(title="Male") +
```

```
    ylim (2,15) +
    annotate("text", x = c('Nov', 'Dec', 'Jan', 'Feb','Mar'), y = 15, label =
c("a", "b", "c", "d", "e"))

# juvenile male body length
mbod <- ggplot(data = jm, mapping = aes(x = Month, y = Length)) +
  geom_jitter(alpha = 0.3) +
  geom_boxplot(alpha = 0) +
  scale_x_discrete(limits=c("Nov", "Dec", "Jan", "Feb", "Mar")) +
  theme_bw () +
  theme(panel.grid = element_blank(),
        axis.title.x=element_blank()) +
  #theme(text = element_text(size=20)) +
  ylab("Body length (mm)") +
    ylim(5, 35) +
    annotate("text", x = c('Nov', 'Dec', 'Jan', 'Feb','Mar'), y = 35, label =
c("a", "a", "b", "c", "d"))

# ----------------
# juvenile females
# ----------------

jf <- dat[dat$Identity == 'f j', ]

jf$Femur <- as.numeric(levels(jf$Femur))[jf$Femur]
jf$Length <- as.numeric(levels(jf$Length))[jf$Length]

# juvenile female femur length
ffem <- ggplot(data = jf, mapping = aes(x = Month, y = Femur)) +
  geom_jitter(alpha = 0.3) +
  geom_boxplot(alpha = 0) +
  scale_x_discrete(limits=c("Nov", "Dec", "Jan", "Feb", "Mar")) +
  theme_bw () +
  theme(panel.grid = element_blank(),
        axis.title.x=element_blank(),
        axis.title.y=element_blank(),
        plot.title = element_text(size = rel(1.05), hjust = 0.5,
        margin = margin(t = 5, b = 5, unit = "pt"))) +
  #theme(text = element_text(size=20)) +
  #ylab("Femur length [mm]") +
    labs(title="Female") +
    ylim(2,15) +
  annotate("text", x = c('Nov', 'Dec', 'Jan', 'Feb','Mar'), y = 15, label =
c("a", "a", "b", "c", "d"))

# juvenile female body length
fbod <- ggplot(data = jf, mapping = aes(x = Month, y = Length)) +
  geom_jitter(alpha = 0.3) +
  geom_boxplot(alpha = 0) +
```

```
  scale_x_discrete(limits=c("Nov", "Dec", "Jan", "Feb", "Mar")) +
  theme_bw () +
  theme(panel.grid = element_blank(),
    axis.title.x=element_blank(),
    axis.title.y=element_blank()) +
  #theme(text = element_text(size=20)) +
  #ylab("Body length [mm]") +
    ylim(5,35) +
    annotate("text", x = c('Nov', 'Dec', 'Jan', 'Feb','Mar'), y = 35, label =
c("a", "b", "c", "d", "e"))

#combine all into the same graph

library(gridExtra)

grid.arrange(mfem, ffem, mbod, fbod, nrow = 2,
             ncol = 2,
             bottom = "Month")
```




```
# ---------------------
# pairwise comparisons
# ---------------------

# junveile male femur
a1 <- aov(jm$Femur ~ jm$Month)
```

```
posthoc <- TukeyHSD(x=a1, 'jm$Month', conf.level=0.95)
print(posthoc)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = jm$Femur ~ jm$Month)
##
## $`jm$Month`
##               diff        lwr         upr     p adj
## Feb-Dec -1.9614325 -2.4455206 -1.47734440 0.0000000
## Jan-Dec -2.5385101 -3.0419772 -2.03504295 0.0000000
## Mar-Dec -1.1057382 -1.6102030 -0.60127349 0.0000000
## Nov-Dec -0.5565657 -1.0874465 -0.02568482 0.0345324
## Jan-Feb -0.5770776 -0.8516314 -0.30252380 0.0000001
## Mar-Feb  0.8556943  0.5793154  1.13207316 0.0000000
## Nov-Feb  1.4048669  1.0827875  1.72694616 0.0000000
## Mar-Jan  1.4327719  1.1237036  1.74184009 0.0000000
## Nov-Jan  1.9819444  1.6314121  2.33247683 0.0000000
## Nov-Mar  0.5491726  0.1972089  0.90113630 0.0002193
```

```
#juvenile female femur
a1 <- aov(jf$Femur ~ jf$Month)
posthoc <- TukeyHSD(x=a1, 'jf$Month', conf.level=0.95)
print(posthoc)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = jf$Femur ~ jf$Month)
##
## $`jf$Month`
##              diff       lwr        upr     p adj
## Feb-Dec -5.809179 -6.833403 -4.7849543 0.0000000
## Jan-Dec -7.018648 -8.052378 -5.9849178 0.0000000
## Mar-Dec -4.041667 -5.075184 -3.0081490 0.0000000
## Nov-Dec -0.254902 -1.342439  0.8326349 0.9681728
## Jan-Feb -1.209469 -1.536550 -0.8823881 0.0000000
## Mar-Feb  1.767512  1.441103  2.0939209 0.0000000
## Nov-Feb  5.554277  5.084042  6.0245111 0.0000000
## Mar-Jan  2.976981  2.621869  3.3320942 0.0000000
## Nov-Jan  6.763746  6.273152  7.2543403 0.0000000
## Nov-Mar  3.786765  3.296618  4.2769109 0.0000000
```

```
#juvenile male body
a1 <- aov(jm$Length ~ jm$Month)
posthoc <- TukeyHSD(x=a1, 'jm$Month', conf.level=0.95)
print(posthoc)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
```

```
##
## Fit: aov(formula = jm$Length ~ jm$Month)
##
## $`jm$Month`
##                diff        lwr        upr     p adj
## Feb-Dec -3.5867769 -4.5996836 -2.5738702 0.0000000
## Jan-Dec -4.9438131 -5.9972686 -3.8903577 0.0000000
## Mar-Dec -1.8027079 -2.8582507 -0.7471651 0.0000357
## Nov-Dec -0.7535354 -1.8643512  0.3572805 0.3426153
## Jan-Feb -1.3570363 -1.9315130 -0.7825595 0.0000000
## Mar-Feb  1.7840689  1.2057733  2.3623645 0.0000000
## Nov-Feb  2.8332415  2.1593223  3.5071607 0.0000000
## Mar-Jan  3.1411052  2.4944104  3.7878000 0.0000000
## Nov-Jan  4.1902778  3.4568233  4.9237323 0.0000000
## Nov-Mar  1.0491726  0.3127232  1.7856220 0.0010163
```

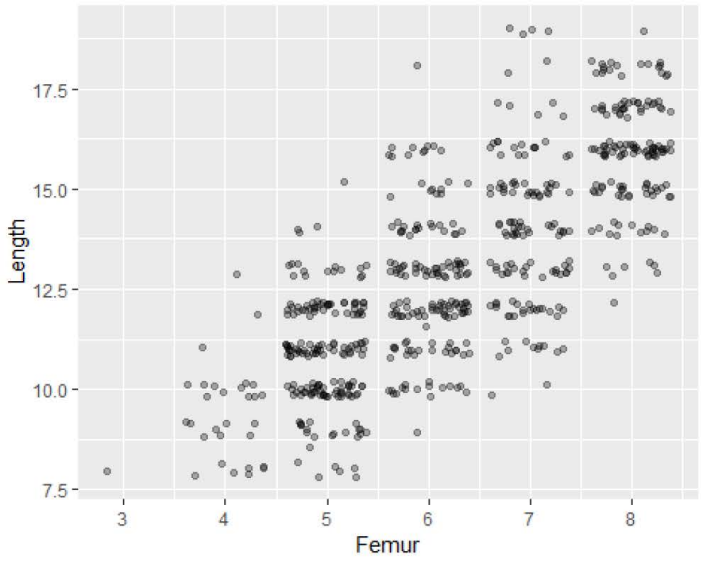
```
#juvenile female body
a1 <- aov(jf$Length ~ jf$Month)
posthoc <- TukeyHSD(x=a1, 'jf$Month', conf.level=0.95)
print(posthoc)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = jf$Length ~ jf$Month)
##
## $`jf$Month`
##                diff        lwr        upr   p adj
## Feb-Dec -14.608696 -16.795937 -12.421454 0.0e+00
## Jan-Dec -17.222999 -19.430540 -15.015458 0.0e+00
## Mar-Dec -11.493056 -13.700143  -9.285968 0.0e+00
## Nov-Dec  -4.287582  -6.610027  -1.965136 5.9e-06
## Jan-Feb  -2.614304  -3.312789  -1.915819 0.0e+00
## Mar-Feb   3.115640   2.418591   3.812689 0.0e+00
## Nov-Feb  10.321114   9.316924  11.325304 0.0e+00
## Mar-Jan   5.729944   4.971597   6.488291 0.0e+00
## Nov-Jan  12.935418  11.887749  13.983086 0.0e+00
## Nov-Mar   7.205474   6.158762   8.252186 0.0e+00
```

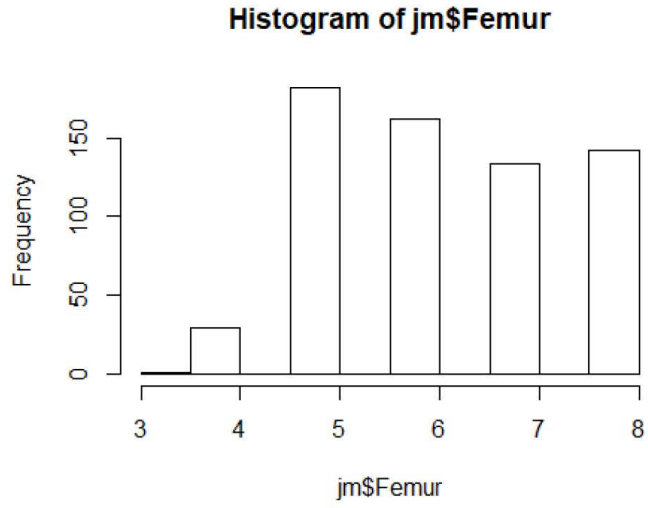
```
# ----------------
# correlation test
# ----------------

library("ggpubr")
```

```
## Loading required package: magrittr
```

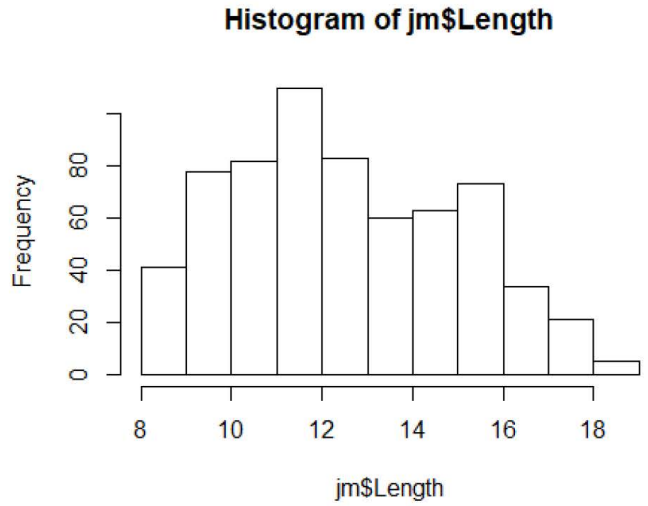
```
# juvenile males

ggplot(jm, aes(x=Femur, y=Length)) +
  geom_jitter(alpha = 0.3)
```



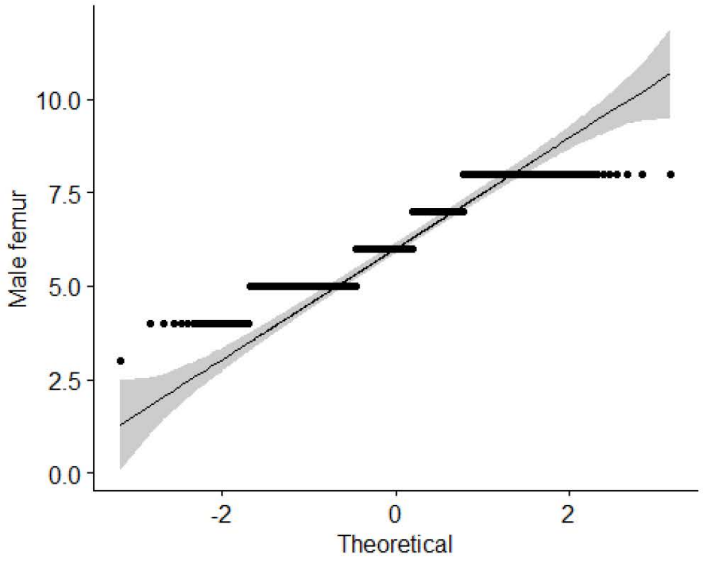

```
shapiro.test(jm$Femur)

##
##  Shapiro-Wilk normality test
##
## data:  jm$Femur
## W = 0.89196, p-value < 2.2e-16

shapiro.test(jm$Length)

##
##  Shapiro-Wilk normality test
##
## data:  jm$Length
## W = 0.96675, p-value = 5.64e-11

hist(jm$Femur)
```



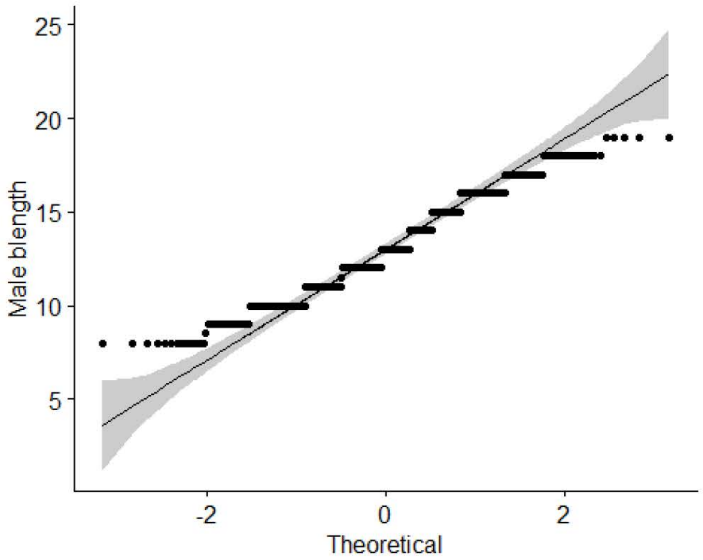

```
hist(jm$Length)
```




```
ggqqplot(jm$Femur, ylab = "Male femur")
```

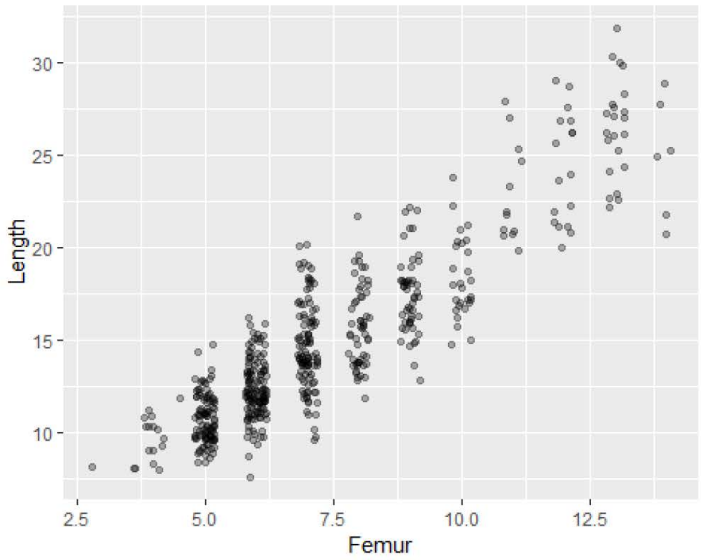



```
ggqqplot(jm$Length, ylab = "Male blength")
```

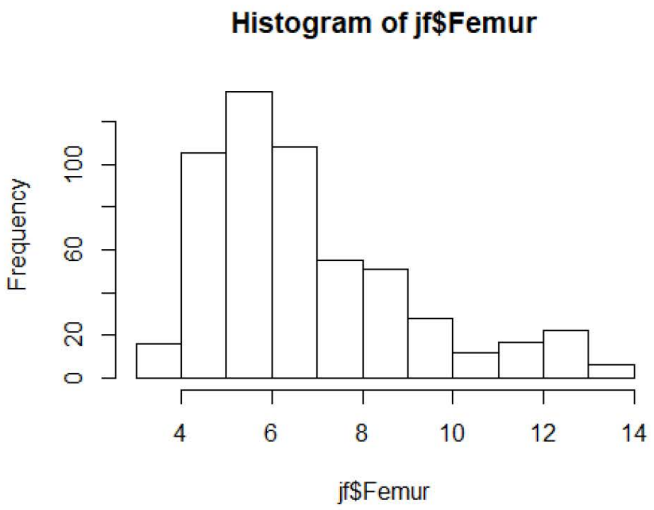



```
cor.test(jm$Femur, jm$Length,  method = "spearman")
```

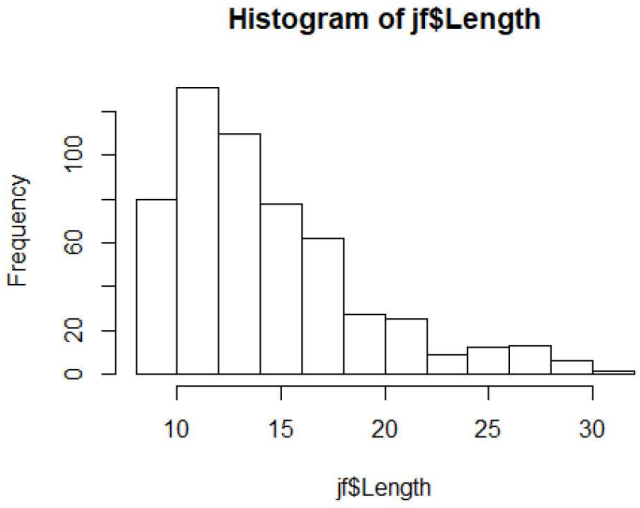
```
##
##  Spearman's rank correlation rho
##
## data:  jm$Femur and jm$Length
## S = 9357400, p-value < 2.2e-16
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.7955585

cor.test(jm$Femur, jm$Length,  method = "kendall")

##
##  Kendall's rank correlation tau
##
## data:  jm$Femur and jm$Length
## z = 22.173, p-value < 2.2e-16
## alternative hypothesis: true tau is not equal to 0
## sample estimates:
##       tau
## 0.6777312

# juvenile females

ggplot(jf, aes(x=Femur, y=Length)) +
  geom_jitter(alpha = 0.3)
```



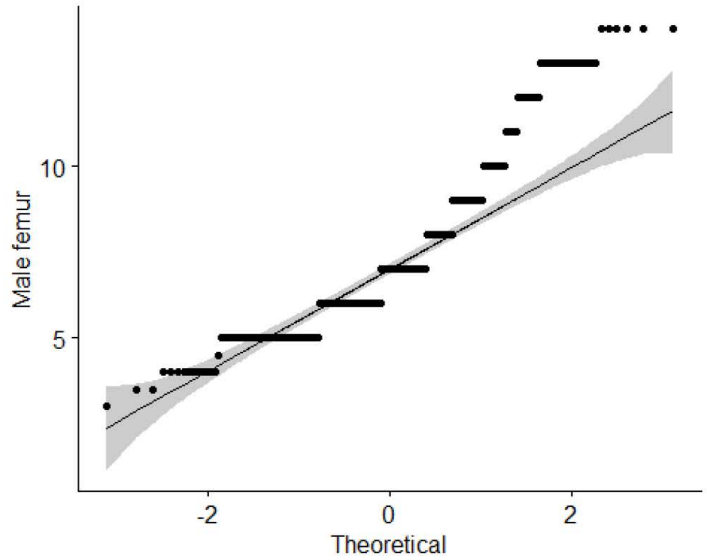
```
shapiro.test(jf$Femur)

##
##  Shapiro-Wilk normality test
##
## data:  jf$Femur
## W = 0.88917, p-value < 2.2e-16

shapiro.test(jf$Length)

##
##  Shapiro-Wilk normality test
##
## data:  jf$Length
## W = 0.89901, p-value < 2.2e-16

hist(jf$Femur)
```



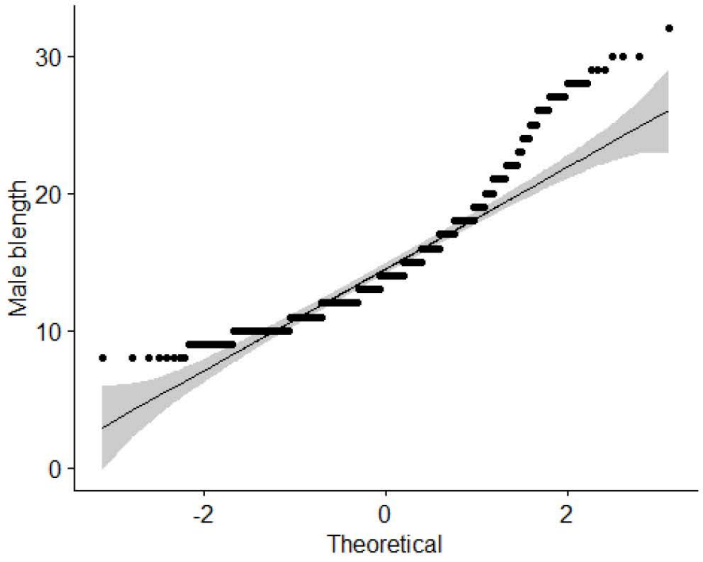

```
hist(jf$Length)
```




```
ggqqplot(jf$Femur, ylab = "Male femur")
```




```
ggqqplot(jf$Length, ylab = "Male blength")
```




```
cor.test(jf$Femur, jf$Length,  method = "spearman")

##
##  Spearman's rank correlation rho
##
## data:  jf$Femur and jf$Length
## S = 3640000, p-value < 2.2e-16
## alternative hypothesis: true rho is not equal to 0
## sample estimates:
##       rho
## 0.8715517

cor.test(jf$Femur, jf$Length,  method = "kendall")

##
##  Kendall's rank correlation tau
##
## data:  jf$Femur and jf$Length
## z = 23.825, p-value < 2.2e-16
## alternative hypothesis: true tau is not equal to 0
## sample estimates:
##      tau
## 0.751738

#-----------------------------------------------------
# probability of detecting an individual within a plot
# -----------------------------------------------------
```

```
#read in dataset
dat <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Pattersons/Full
removal study dataset_FIXED.csv')

str(dat)
```

```
## 'data.frame':    1481 obs. of  38 variables:
##  $ Checked         : Factor w/ 2 levels "","x": 2 2 2 2 2 2 2 2 2 ...
##  $ Event           : int  1 2 3 4 5 6 7 8 9 10 ...
##  $ Date            : Factor w/ 54 levels "1/02/2016","1/03/2016",..: 3 3 3
3 3 3 3 24 24 24 ...
##  $ Month           : Factor w/ 5 levels "Dec","Feb","Jan",..: 5 5 5 5 5 5
5 5 5 5 ...
##  $ Sampling.Session: int  1 1 1 1 1 1 1 1 1 1 ...
##  $ Air             : Factor w/ 62 levels "","10.3","11.4",..: 62 62 62 62
62 62 62 62 62 62 ...
##  $ Ground          : Factor w/ 58 levels "","14.1","14.4",..: 58 58 58 58
58 58 58 58 58 58 ...
##  $ Baro            : Factor w/ 59 levels "","960.6","971.5",..: 59 59 59
59 59 59 59 59 59 59 ...
##  $ Baro.direction  : Factor w/ 8 levels "","down","down right",..: 4 4 4 4
4 4 4 4 4 4 ...
##  $ Sky             : Factor w/ 5 levels "","clear","high cloud",..: 2 2 2
2 2 2 2 2 2 2 ...
##  $ Wind            : Factor w/ 8 levels "","breeze","cold",..: 2 2 2 2 2 2
2 7 7 7 ...
##  $ Start.time      : Factor w/ 173 levels "","1","1.02",..: 19 19 19 19
108 127 127 42 42 42 ...
##  $ End.time        : Factor w/ 210 levels "","1.04","1.06",..: 110 110 110
110 141 160 160 80 80 80 ...
##  $ Plot            : Factor w/ 6 levels "A 0-40","A 60-100",..: 4 4 4 4 4
4 4 5 5 5 ...
##  $ First.transect  : Factor w/ 2 levels "No","Yes": 1 1 1 1 1 1 1 2 2 1
...
##  $ Pass            : Factor w/ 6 levels "A","B","C","D",..: 1 1 1 1 2 3 3
1 1 1 ...
##  $ Number          : int  2 3 4 5 2 1 2 1 2 3 ...
##  $ Sex             : Factor w/ 3 levels "","f","m": 3 2 3 2 3 2 2 3 3 2
...
##  $ Length          : Factor w/ 36 levels "10","11","11.5",..: 6 15 8 13 5
14 10 7 8 8 ...
##  $ Femur           : Factor w/ 19 levels "10","11","12",..: 16 4 16 2 16 3
1 16 17 17 ...
##  $ Adult..Juv      : Factor w/ 3 levels "","a","j": 3 3 3 3 3 3 3 3 3 3
...
##  $ Identity        : Factor w/ 8 levels "f ","f a","f j",..: 6 3 6 3 6 3 3
6 6 3 ...
##  $ Colour          : Factor w/ 37 levels "","," grey","," grey mottled",..: 21
8 21 21 33 19 21 8 8 32 ...
```

```
##  $ Location        : num  76 90 91.5 92 99 73 97 39.5 33 1 ...
##  $ South           : Factor w/ 272 levels "44","44.0417",..: 236 234 234
234 230 237 231 217 214 202 ...
##  $ East            : Factor w/ 216 levels "170.42","170.422369",..: 181
180 177 179 176 179 178 194 198 213 ...
##  $ Elevation       : Factor w/ 37 levels "",".","15","659",..: 22 24 21 22
22 24 23 27 27 26 ...
##  $ Notes           : Factor w/ 54 levels "","0 nymphs",..: 1 1 1 1 1 1 43
1 1 40 ...
##  $ Time            : num  NA NA NA NA NA NA NA NA NA NA ...
##  $ X               : num  NA NA NA NA NA NA NA NA NA NA ...
##  $ Cloud           : Factor w/ 11 levels "","971.5","978.6",..: 1 1 1 1 1
1 1 1 1 1 ...
##  $ Wind.1          : Factor w/ 8 levels "","breeze","down",..: 1 1 1 1 1 1
1 1 1 1 ...
##  $ Baro.1          : Factor w/ 8 levels "","978.8","979.4",..: 1 1 1 1 1 1
1 1 1 1 ...
##  $ Direction       : Factor w/ 6 levels "","breeze","down",..: 1 1 1 1 1 1
1 1 1 1 ...
##  $ Air.1           : num  NA NA NA NA NA NA NA NA NA NA ...
##  $ G..Shade        : num  NA NA NA NA NA NA NA NA NA NA ...
##  $ G..Sun          : num  NA NA NA NA NA NA NA NA NA NA ...
##  $ Notes.1         : logi  NA NA NA NA NA NA ...

# --------------
# clean the data
# --------------

#remove columns that aren't of interest
library(dplyr)

##
## Attaching package: 'dplyr'

## The following object is masked from 'package:gridExtra':
##
##     combine

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union

dat <- dat %>%
  select_("Month", "Sampling.Session","Air", "Ground", "Sky", "Plot", "Pass",
"Sex", "Length", "Identity", "First.transect")

#replace "no data" and " " with "NA"
```

```
dat[dat == "no data" | dat == ""] <- NA

#make a new column containing a success/fail identifier for individuals found
on the first search of an area or not
dat$Detect <- (dat$Pass == 'A')
dat$Detect <- as.integer(as.logical(dat$Detect))

#convert length from integer to numeric
dat$Length <- as.numeric(as.character(dat$Length))

#drop rows where 'Sky' is NA. That way all models have the same number of
data rows
library(tidyr)

##
## Attaching package: 'tidyr'

## The following object is masked from 'package:magrittr':
##
##     extract

dat <- dat %>%
  drop_na(Sky) %>%
  drop_na(Length)

#drop rows where grasshoppers were found on the first transect search of a
plot
dat <-dat[!(dat$First.transect=="Yes"),]

#drop levels from the data
dat <- droplevels(dat)

#create a new columns to use for observation level random intercept
dat$Eps <- 1:nrow(dat)

# --------------------
# Statistical analysis
# --------------------

library(lme4)

## Loading required package: Matrix

##
## Attaching package: 'Matrix'

## The following object is masked from 'package:tidyr':
##
##     expand

library(lmerTest)
```

```
##
## Attaching package: 'lmerTest'

## The following object is masked from 'package:lme4':
##
##     lmer

## The following object is masked from 'package:stats':
##
##     step

lmerControl("bobyqa")

## $optimizer
## [1] "bobyqa"
##
## $restart_edge
## [1] TRUE
##
## $boundary.tol
## [1] 1e-05
##
## $calc.derivs
## [1] TRUE
##
## $use.last.params
## [1] FALSE
##
## $checkControl
## $checkControl$check.nobs.vs.rankZ
## [1] "ignore"
##
## $checkControl$check.nobs.vs.nlev
## [1] "stop"
##
## $checkControl$check.nlev.gtreq.5
## [1] "ignore"
##
## $checkControl$check.nlev.gtr.1
## [1] "stop"
##
## $checkControl$check.nobs.vs.nRE
## [1] "stop"
##
## $checkControl$check.rankX
## [1] "message+drop.cols"
##
## $checkControl$check.scaleX
## [1] "warning"
##
## $checkControl$check.formula.LHS
```

```
## [1] "stop"
##
##
## $checkConv
## $checkConv$check.conv.grad
## $checkConv$check.conv.grad$action
## [1] "warning"
##
## $checkConv$check.conv.grad$tol
## [1] 0.002
##
## $checkConv$check.conv.grad$relTol
## NULL
##
##
## $checkConv$check.conv.singular
## $checkConv$check.conv.singular$action
## [1] "message"
##
## $checkConv$check.conv.singular$tol
## [1] 1e-04
##
##
## $checkConv$check.conv.hess
## $checkConv$check.conv.hess$action
## [1] "warning"
##
## $checkConv$check.conv.hess$tol
## [1] 1e-06
##
##
##
## $optCtrl
## list()
##
## attr(,"class")
## [1] "lmerControl" "merControl"

#all models converge now that i've added the random intercept for each
observation

mod1 <- glmer(Detect ~ Length + Sex*Month + Sky + (1|Plot) + (1|Eps), family
= binomial, data = dat)

## singular fit

mod1a <- glmer(Detect ~ Length + Sex*Month + Sky + (1|Plot), family =
binomial, data = dat)

anova(mod1, mod1a) #no sig diff, mod1a better
```

```
## Data: dat
## Models:
## mod1a: Detect ~ Length + Sex * Month + Sky + (1 | Plot)
## mod1: Detect ~ Length + Sex * Month + Sky + (1 | Plot) + (1 | Eps)
##       Df  AIC    BIC  logLik deviance Chisq Chi Df Pr(>Chisq)
## mod1a 15 1829 1907.1 -899.49     1799
## mod1  16 1831 1914.3 -899.49     1799     0      1     0.9987

# -----

mod2 <- glmer(Detect ~ Length + Sex + Month + Sky + (1|Plot) + (1|Eps),
family = binomial, data = dat)

## singular fit

mod2a <- glmer(Detect ~ Length + Sex + Month + Sky + (1|Plot), family =
binomial, data = dat)

anova(mod2, mod2a) #no sig diff, mod2a better

## Data: dat
## Models:
## mod2a: Detect ~ Length + Sex + Month + Sky + (1 | Plot)
## mod2: Detect ~ Length + Sex + Month + Sky + (1 | Plot) + (1 | Eps)
##       Df    AIC    BIC  logLik deviance Chisq Chi Df Pr(>Chisq)
## mod2a 11 1823.1 1880.4 -900.55   1801.1
## mod2  12 1825.1 1887.6 -900.55   1801.1     0      1     0.9999

# ----

mod3 <- glmer(Detect ~ Length + Month + Sex + (1|Plot), family = binomial,
data = dat)

mod4 <- glmer(Detect ~ Length + Month + (1|Plot), family = binomial, data =
dat)

mod5 <- glmer(Detect ~ Length + (1|Plot), family = binomial, data = dat)

mod6 <- glmer(Detect ~ Month + (1|Plot), family = binomial, data = dat)

anova(mod1a, mod2a) # no sig diff, mod2a best

## Data: dat
## Models:
## mod2a: Detect ~ Length + Sex + Month + Sky + (1 | Plot)
## mod1a: Detect ~ Length + Sex * Month + Sky + (1 | Plot)
##       Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## mod2a 11 1823.1 1880.4 -900.55   1801.1
## mod1a 15 1829.0 1907.1 -899.49   1799.0 2.1209      4     0.7135

anova(mod2a, mod3) # no sig diff, mod3 best
```

```
## Data: dat
## Models:
## mod3: Detect ~ Length + Month + Sex + (1 | Plot)
## mod2a: Detect ~ Length + Sex + Month + Sky + (1 | Plot)
##       Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## mod3   8 1817.4 1859.0 -900.68   1801.4
## mod2a 11 1823.1 1880.4 -900.55   1801.1 0.2495      3     0.9692
```

```
anova(mod3, mod4) # no sig diff, mod4 best
```

```
## Data: dat
## Models:
## mod4: Detect ~ Length + Month + (1 | Plot)
## mod3: Detect ~ Length + Month + Sex + (1 | Plot)
##      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## mod4  7 1817.2 1853.7 -901.63   1803.2
## mod3  8 1817.4 1859.0 -900.68   1801.4 1.8979      1     0.1683
```

```
anova(mod4, mod5) # sig diff, mod4 best
```

```
## Data: dat
## Models:
## mod5: Detect ~ Length + (1 | Plot)
## mod4: Detect ~ Length + Month + (1 | Plot)
##      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## mod5  3 1821.0 1836.7 -907.51   1815.0
## mod4  7 1817.2 1853.7 -901.63   1803.2 11.771      4    0.01914 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(mod4, mod6) # sig diff, mod4 best
```

```
## Data: dat
## Models:
## mod6: Detect ~ Month + (1 | Plot)
## mod4: Detect ~ Length + Month + (1 | Plot)
##      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## mod6  6 1832.7 1863.9 -910.32   1820.7
## mod4  7 1817.2 1853.7 -901.63   1803.2 17.393      1  3.039e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
AIC(mod1, mod2, mod3, mod4, mod5, mod6)
```

```
##      df      AIC
## mod1 16 1830.986
## mod2 12 1825.107
## mod3  8 1817.356
## mod4  7 1817.254
## mod5  3 1821.025
## mod6  6 1832.647
```

```r
# mod4, lowest AIC

#check for overdipsersion
E1 <- residuals(mod4)
p1 <- length(fixef(mod4)) +1
(overdisp1 <- sum(E1^2) / nrow(dat) - p1)
```

```
## [1] -5.675199
```

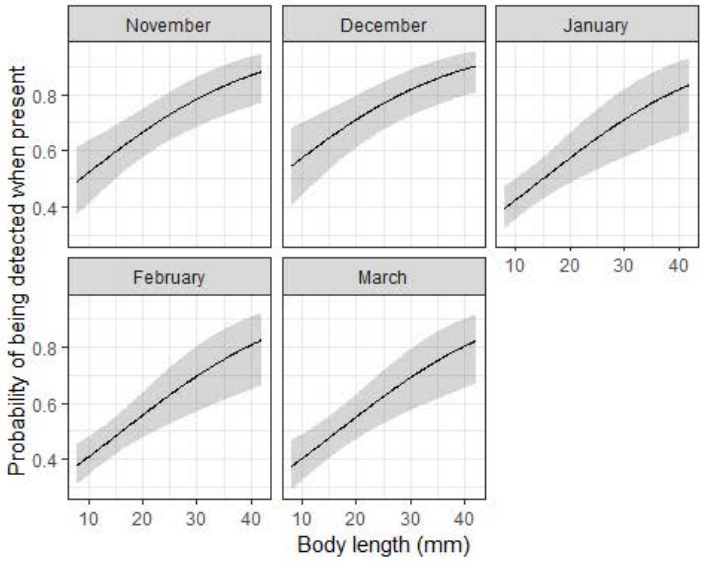
```r
#check summary
summary(mod4)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: binomial  ( logit )
## Formula: Detect ~ Length + Month + (1 | Plot)
##    Data: dat
##
##      AIC      BIC   logLik deviance df.resid
##   1817.3   1853.7   -901.6   1803.3     1345
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.2896 -0.9199  0.4587  0.9491  1.4179
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  Plot   (Intercept) 0.0529   0.23
## Number of obs: 1352, groups:  Plot, 6
##
## Fixed effects:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) -0.29869    0.37876  -0.789  0.43035
## Length       0.06008    0.01507   3.987  6.7e-05 ***
## MonthFeb    -0.67290    0.25265  -2.663  0.00774 **
## MonthJan    -0.59933    0.26667  -2.247  0.02461 *
## MonthMar    -0.69561    0.25069  -2.775  0.00552 **
## MonthNov    -0.21161    0.27537  -0.768  0.44222
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr) Length MnthFb MnthJn MnthMr
## Length   -0.789
## MonthFeb -0.773  0.381
## MonthJan -0.789  0.431  0.838
## MonthMar -0.668  0.246  0.808  0.781
## MonthNov -0.501  0.090  0.683  0.651  0.672
```

```r
# --------------
# Plot for mod4
```

```
# --------------

#note: make sure the order of the months is alphabetical as it is specified
in the model.
NewData <- expand.grid(Month = c("Dec", "Feb", "Jan", "Mar", "Nov"),
                       Length = seq(8, 42, length=200),
                       Plot    = levels(dat$Plot))

head(NewData)

##   Month   Length   Plot
## 1   Dec 8.000000 A 0-40
## 2   Feb 8.000000 A 0-40
## 3   Jan 8.000000 A 0-40
## 4   Mar 8.000000 A 0-40
## 5   Nov 8.000000 A 0-40
## 6   Dec 8.170854 A 0-40

X <- model.matrix(~ Length + Month,
                  data = NewData)

NewData$eta         <- X %*% fixef(mod4)
NewData$SuccessPred <- exp(NewData$eta) / (1 + exp(NewData$eta))
head(NewData,15)

##    Month   Length   Plot          eta SuccessPred
## 1    Dec 8.000000 A 0-40  0.181944982   0.5453612
## 2    Feb 8.000000 A 0-40 -0.490955606   0.3796685
## 3    Jan 8.000000 A 0-40 -0.417380603   0.3971437
## 4    Mar 8.000000 A 0-40 -0.513663465   0.3743351
## 5    Nov 8.000000 A 0-40 -0.029660650   0.4925854
## 6    Dec 8.170854 A 0-40  0.192209747   0.5479050
## 7    Feb 8.170854 A 0-40 -0.480690842   0.3820890
## 8    Jan 8.170854 A 0-40 -0.407115839   0.3996039
## 9    Mar 8.170854 A 0-40 -0.503398701   0.3767423
## 10   Nov 8.170854 A 0-40 -0.019395886   0.4951512
## 11   Dec 8.341709 A 0-40  0.202474511   0.5504464
## 12   Feb 8.341709 A 0-40 -0.470426077   0.3845154
## 13   Jan 8.341709 A 0-40 -0.396851074   0.4020691
## 14   Mar 8.341709 A 0-40 -0.493133936   0.3791556
## 15   Nov 8.341709 A 0-40 -0.009131121   0.4977172

#Get the standard errors
NewData$VarPred <- diag(X %*% vcov(mod4) %*% t(X))
NewData$sePred  <- sqrt(NewData$VarPred)

NewData$seLow  <- exp(NewData$eta - 1.96 * NewData$sePred) / (1 +
exp(NewData$eta - 1.96 * NewData$sePred))
NewData$seHigh <- exp(NewData$eta + 1.96 * NewData$sePred) / (1 +
```

```
exp(NewData$eta + 1.96 * NewData$sePred))
head(NewData, 10)

##    Month   Length   Plot         eta SuccessPred    VarPred    sePred
## 1    Dec 8.000000 A 0-40  0.18194498   0.5453612 0.08598508 0.2932321
## 2    Feb 8.000000 A 0-40 -0.49095561   0.3796685 0.02500793 0.1581390
## 3    Jan 8.000000 A 0-40 -0.41738060   0.3971437 0.02549883 0.1596835
## 4    Mar 8.000000 A 0-40 -0.51366347   0.3743351 0.03681160 0.1918635
## 5    Nov 8.000000 A 0-40 -0.02966065   0.4925854 0.06316640 0.2513293
## 6    Dec 8.170854 A 0-40  0.19220975   0.5479050 0.08507458 0.2916755
## 7    Feb 8.170854 A 0-40 -0.48069084   0.3820890 0.02459327 0.1568224
## 8    Jan 8.170854 A 0-40 -0.40711584   0.3996039 0.02518063 0.1586841
## 9    Mar 8.170854 A 0-40 -0.50339870   0.3767423 0.03621878 0.1903123
## 10   Nov 8.170854 A 0-40 -0.01939589   0.4951512 0.06238326 0.2497664
##        seLow    seHigh
## 1  0.4030458 0.6806325
## 2  0.3098313 0.4548723
## 3  0.3251157 0.4739235
## 4  0.2911685 0.4656514
## 5  0.3723225 0.6137125
## 6  0.4062537 0.6821985
## 7  0.3125848 0.4567784
## 8  0.3278034 0.4759947
## 9  0.2939221 0.4674495
## 10 0.3754425 0.6154184

#reorder the levels so that they plto correctly
NewData$Month2 <- factor(NewData$Month, levels = c("Nov", "Dec", "Jan",
"Feb", "Mar"))

#rename the Months to be full name
levels(NewData$Month2)[levels(NewData$Month2)=="Nov"] <- "November"
levels(NewData$Month2)[levels(NewData$Month2)=="Dec"] <- "December"
levels(NewData$Month2)[levels(NewData$Month2)=="Jan"] <- "January"
levels(NewData$Month2)[levels(NewData$Month2)=="Feb"] <- "February"
levels(NewData$Month2)[levels(NewData$Month2)=="Mar"] <- "March"

library(ggplot2)

ggplot(data = NewData, aes(Length, SuccessPred)) +
  #geom_point() + #plot the points
  ylab("Probability of being detected when present") +
  xlab("Body length (mm)") +
  theme_bw() +
  geom_line(data = NewData, aes(y=SuccessPred)) +
  geom_ribbon(aes(ymin=seLow, ymax=seHigh, colour=NULL), alpha=0.2) +
  facet_wrap(vars(Month2))
```


November
December
January
February
March
Probability of being detected when present
0.8
0.6
0.4
10
20
30
40
Body length (mm)

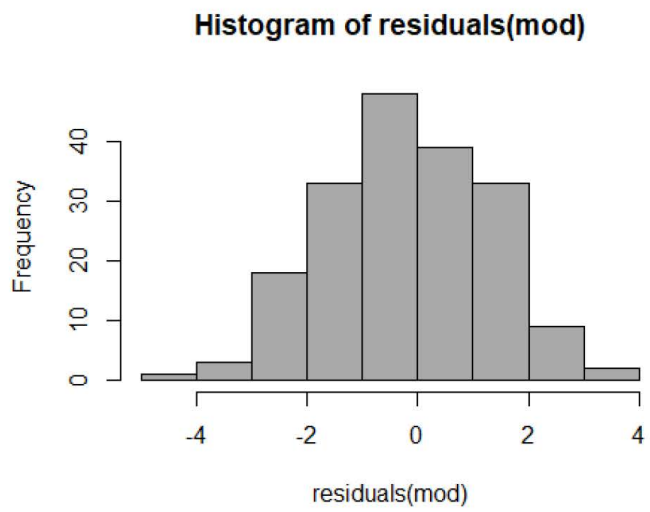
Appendix H

Chapter 6: Statistical analyses and R code

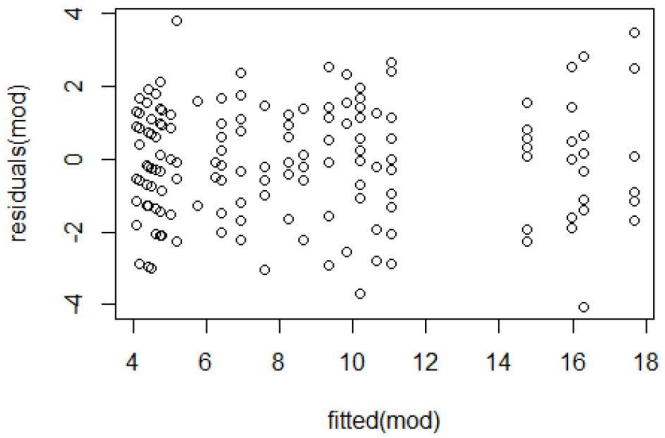
Chapter 6: Designing monitoring protocols to measure conservation benefits for a highly cryptic threatened grasshopper

```
# ------------------------------------------------------
# compare grasshoppers from transects and plot searches
# ------------------------------------------------------

# ----------------
# FULL POPULATION
# ----------------

# read in the data
dat <- read.csv("C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Nov 18 Analysis/Density_full_popn_Patersons_Transect_v_Plot.csv")

# clean the data
dat <- dat[dat$Month != 'Apr',]
dat <- droplevels(dat)
dat$Method <- as.factor(dat$Method)
dat$Season <- as.factor(dat$Season)
dat$Name <- as.factor(dat$Name)

# Modelling

library(lme4)

## Loading required package: Matrix

library(lmerTest)

##
## Attaching package: 'lmerTest'

## The following object is masked from 'package:lme4':
##
##     lmer

## The following object is masked from 'package:stats':
##
##     step

# POISSON MODEL ----

mod <- glm(total ~ Method + Month*Season, family = poisson, data = dat)

# check model fit
```

```r
#check for normal distribution of residuals
hist(residuals(mod),col="darkgray")
```

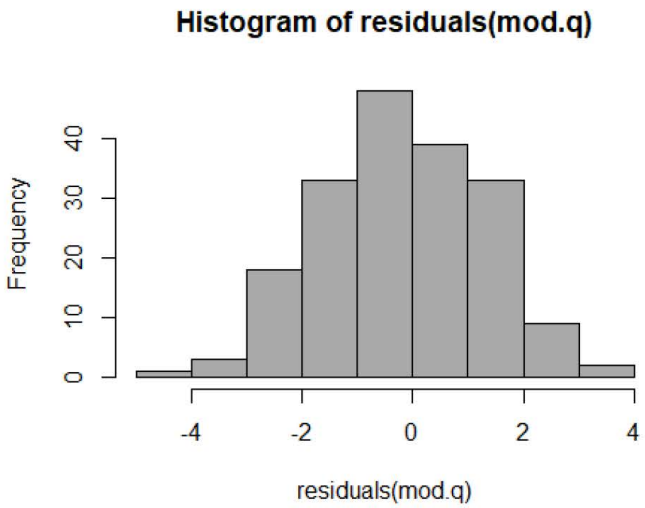



```r
#check for homoscedastic residulas
plot(fitted(mod),residuals(mod))
```

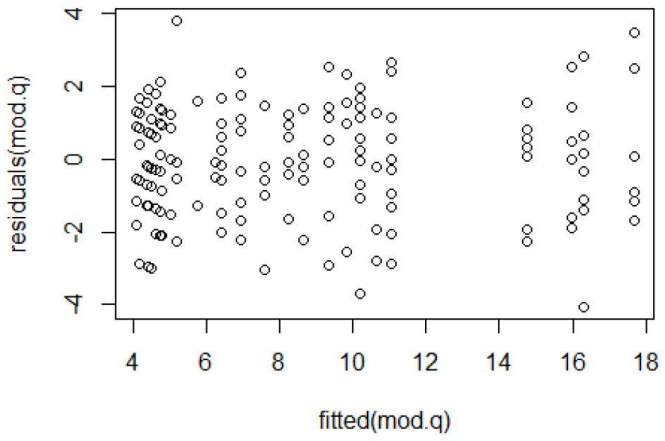



```
#If p> 0.05, then model fit is good.
1 - pchisq(summary(mod)$deviance, summary(mod)$df.residual)

## [1] 0

#Look at the critical level of dispersion by comparing to the degrees of
freedom
qchisq(0.95, df.residual(mod))

## [1] 201.4234

#get model deviance
deviance(mod)

## [1] 398.4886

#get pearson's chi2
pr <- residuals(mod,"pearson")
sum(pr^2) #if values are lower than critical value, then model fit is good.

## [1] 374.7273

#this model is not a good fit and the data are over dispersed.

# QUASI POISSON MODEL ----

mod.q <- glm(total ~ Method + Month*Season, family = quasipoisson, data =
dat)
```

```
# check model fit

#check for normal distribution of residuals
hist(residuals(mod.q),col="darkgray")
```

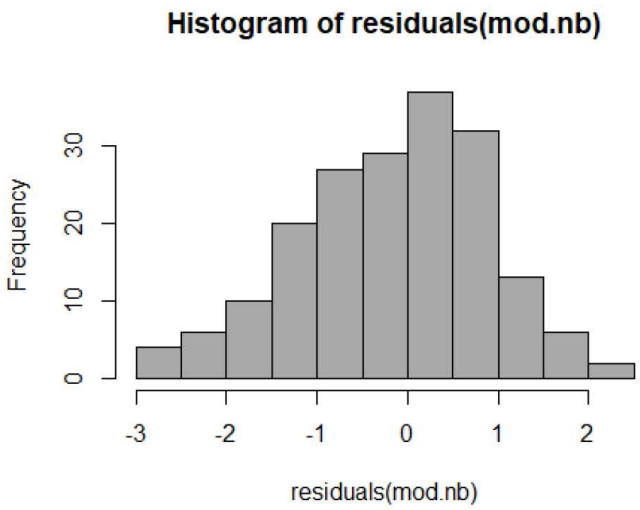



```
#check for homoscedastic residulas
plot(fitted(mod.q),residuals(mod.q))
```

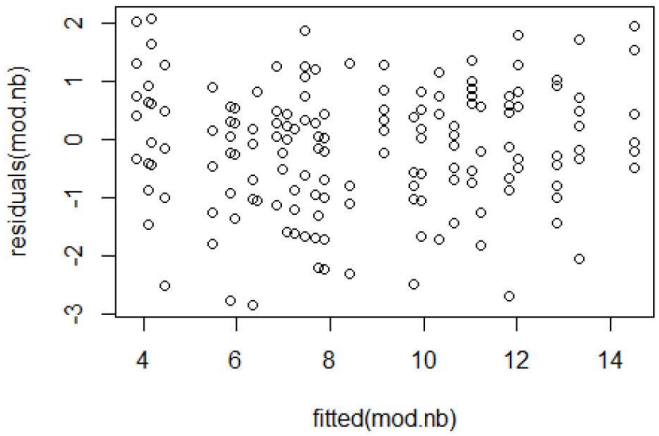



```
#If p> 0.05, then model fit is good.
1 - pchisq(summary(mod.q)$deviance, summary(mod.q)$df.residual)

## [1] 0

#Look at the critical level of dispersion by comparing to the degrees of
freedom
qchisq(0.95, df.residual(mod.q))

## [1] 201.4234

#get model deviance
deviance(mod.q)

## [1] 398.4886

#get pearson's chi2
pr <- residuals(mod.q,"pearson")
sum(pr^2)#if values are lower than critical value, then model fit is good.

## [1] 374.7273

#this model is still not a good fit and the data are over-dispersed.

# NEGATIVE BINOMIAL MODEL ----

library(MASS)
```

```
mod.nb <- glm.nb(total ~ Method + Month + Season, data = dat)

#check for normal distribution of residuals
hist(residuals(mod.nb),col="darkgray")
```




```
#check for homoscedastic residuals
plot(fitted(mod.nb),residuals(mod.nb))
```




```
#If p> 0.05, then model fit is good.
1 - pchisq(summary(mod.nb)$deviance, summary(mod.nb)$df.residual)

## [1] 0.0923454

#look at the critical level of dispersion by comparing to the degrees of
freedom
qchisq(0.95, df.residual(mod.nb))

## [1] 210.1298

#get model deviance
deviance(mod.nb)

## [1] 203.4926

#get pearson's chi2
pr <- residuals(mod.nb,"pearson")
sum(pr^2)

## [1] 174.3532

# if values are lower than critical value, then model fit is good.

# Pearson's is lower than critical, but the other tests of fit still aren't
that great.
# Using an interaction model between month and season does not fit the data
well, even though it has a lower AIC value (not much of a difference though).
```

```r
# The best fitting model with reasonable low AIC is a negative binomial model
with no interaction and all the variables included.

summary(mod.nb)
```

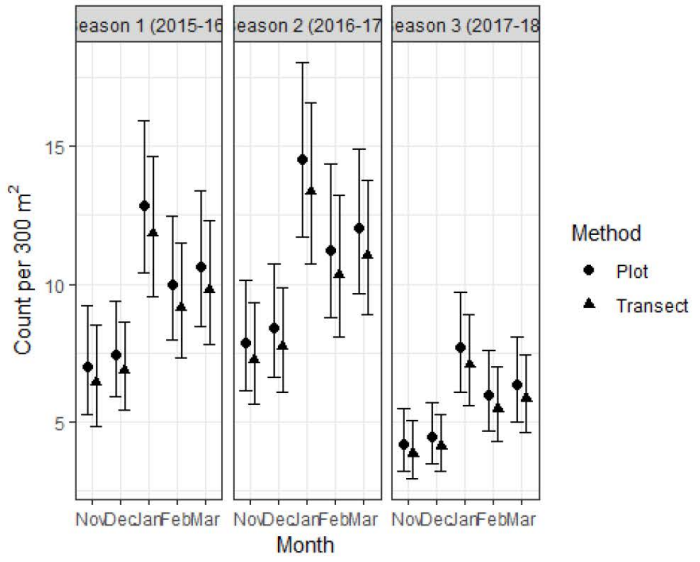
```
##
## Call:
## glm.nb(formula = total ~ Method + Month + Season, data = dat,
##     init.theta = 4.81803991, link = log)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -2.84679  -0.88438  -0.08078   0.56527   2.07353
##
## Coefficients:
##                Estimate Std. Error z value Pr(>|z|)
## (Intercept)     2.00813    0.11797  17.023  < 2e-16 ***
## MethodTransect -0.08372    0.08472  -0.988  0.32302
## MonthFeb        0.28937    0.13731   2.107  0.03509 *
## MonthJan        0.54572    0.12974   4.206 2.60e-05 ***
## MonthMar        0.35664    0.13347   2.672  0.00754 **
## MonthNov       -0.06529    0.15229  -0.429  0.66810
## Season2         0.12123    0.10204   1.188  0.23481
## Season3        -0.51571    0.10840  -4.758 1.96e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for Negative Binomial(4.818) family taken to be 1)
##
##     Null deviance: 279.68  on 185  degrees of freedom
## Residual deviance: 203.49  on 178  degrees of freedom
## AIC: 1093.7
##
## Number of Fisher Scoring iterations: 1
##
##
##               Theta:  4.818
##           Std. Err.:  0.841
##
##  2 x log-likelihood:  -1075.725
```

```r
# ------------------------
# predict the means values
# ------------------------

library(dplyr)
```

```
##
## Attaching package: 'dplyr'
```

```
## The following object is masked from 'package:MASS':
##
##     select

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union

#make new dataset
nd <- expand.grid(Method = levels(dat$Method),
                  Month = levels(dat$Month),
                  Season = levels(dat$Season))

#print out the means
cbind(nd,
      Mean = predict(mod.nb, newdata=nd, type="response"),
      SE = predict(mod.nb, newdata=nd, type="response", se.fit=T)$se.fit
)

##      Method Month Season      Mean        SE
## 1      Plot   Dec      1  7.449383 0.8787754
## 2  Transect   Dec      1  6.851093 0.8117103
## 3      Plot   Feb      1  9.949253 1.1443125
## 4  Transect   Feb      1  9.150188 1.0564123
## 5      Plot   Jan      1 12.856558 1.4000227
## 6  Transect   Jan      1 11.823996 1.2921414
## 7      Plot   Mar      1 10.641555 1.2395507
## 8  Transect   Mar      1  9.786889 1.1440377
## 9      Plot   Nov      1  6.978522 0.9982208
## 10 Transect   Nov      1  6.418049 0.9208702
## 11     Plot   Dec      2  8.409519 1.0465378
## 12 Transect   Dec      2  7.734117 0.9659990
## 13     Plot   Feb      2 11.231593 1.4002167
## 14 Transect   Feb      2 10.329538 1.2916015
## 15     Plot   Jan      2 14.513615 1.6047675
## 16 Transect   Jan      2 13.347968 1.4804724
## 17     Plot   Mar      2 12.013125 1.3319389
## 18 Transect   Mar      2 11.048302 1.2293604
## 19     Plot   Nov      2  7.877971 1.0104668
## 20 Transect   Nov      2  7.245259 0.9326373
## 21     Plot   Dec      3  4.447830 0.5695850
## 22 Transect   Dec      3  4.090607 0.5263556
## 23     Plot   Feb      3  5.940437 0.7430875
## 24 Transect   Feb      3  5.463336 0.6864043
## 25     Plot   Jan      3  7.676312 0.9078135
## 26 Transect   Jan      3  7.059797 0.8385024
## 27     Plot   Mar      3  6.353792 0.7710213
```

```
## 28 Transect   Mar      3  5.843494 0.7122935
## 29     Plot   Nov      3  4.166692 0.5718371
## 30 Transect   Nov      3  3.832048 0.5281759

newY <- predict(mod.nb, newdata = nd, type = "link", se.fit = TRUE)

pd <- data.frame(nd, total = newY)
pd <- mutate(pd, total=exp(total.fit))
pd <- mutate(pd, ucl=exp(total.fit + 1.96*total.se.fit))
pd <- mutate(pd, lcl=exp(total.fit - 1.96*total.se.fit))


# Graph the output

library(ggplot2)

levels(pd$Season) <- c("Season 1 (2015-16)", "Season 2 (2016-17)", "Season 3
(2017-18)")

#reorder the months
pd$Month <- factor(pd$Month, levels=c("Nov", "Dec", "Jan", "Feb", "Mar"))

ggplot(pd, aes(x = Month, y = total, ymax=ucl, ymin=lcl, shape = Method)) +
  geom_point(position=position_dodge(width=0.6), size = 2) +
  geom_errorbar(position=position_dodge(width=0.6))+
  theme_bw() +
  facet_wrap(~ Season) +
  labs(x = "Month", y = expression ("Count per 300"~m^2))
```




```
# ------------
# ADULT FEMALE
# ------------

# read in the data
af <- read.csv("C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Nov 18
Analysis/Density_adult_female_plot_v_transect_patersons.csv")

library(dplyr)
str(af)
```

```
## 'data.frame':    188 obs. of  7 variables:
##  $ X               : int  1 2 3 4 5 6 7 8 9 10 ...
##  $ Month           : Factor w/ 6 levels "Apr","Dec","Feb",..: 6 6 2 2 2 2
2 2 2 2 ...
##  $ Sampling.session: int  1 2 3 4 5 6 7 8 9 10 ...
##  $ total           : int  1 0 2 0 0 3 1 0 1 0 ...
##  $ Name            : Factor w/ 6 levels "Plot_S1_300",..: 1 1 1 1 1 1 1 1
1 1 ...
##  $ Method          : Factor w/ 2 levels "Plot","Transect": 1 1 1 1 1 1 1 1
1 1 ...
##  $ Season          : int  1 1 1 1 1 1 1 1 1 1 ...
```

```
af <- af %>%
  filter(Month == "Nov" | Month == "Dec")
```

```r
af$Method <- as.factor(as.character(af$Method))
af$Season <- as.factor(as.character(af$Season))
af <- droplevels(af)

# POISSON MODEL ----

library(lme4)
library(lmerTest)

#first model
mod <- glm(total ~ Method + Month + Season, data = af, family = poisson)

# check significance of month and season variables

mod2 <- glm(total ~ Method + Month, data = af, family = poisson)

mod3 <- glm(total ~ Method + Season, data = af, family = poisson)

mod4 <- glm(total ~ Method, data = af, family = poisson)

anova(mod, mod2, test = "Chisq")

## Analysis of Deviance Table
##
## Model 1: total ~ Method + Month + Season
## Model 2: total ~ Method + Month
##   Resid. Df Resid. Dev Df Deviance Pr(>Chi)
## 1        63     66.572
## 2        65     78.774 -2  -12.202  0.00224 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

anova(mod, mod3, test = "Chisq")

## Analysis of Deviance Table
##
## Model 1: total ~ Method + Month + Season
## Model 2: total ~ Method + Season
##   Resid. Df Resid. Dev Df Deviance Pr(>Chi)
## 1        63     66.572
## 2        64     68.678 -1  -2.1057   0.1468

anova(mod, mod4, test = "Chisq")

## Analysis of Deviance Table
##
## Model 1: total ~ Method + Month + Season
## Model 2: total ~ Method
##   Resid. Df Resid. Dev Df Deviance Pr(>Chi)
## 1        63     66.572
```

```
## 2        66     79.054 -3  -12.482 0.005901 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
AIC(mod, mod2, mod3, mod4)
```

```
##      df      AIC
## mod   5 130.8515
## mod2  3 139.0537
## mod3  4 130.9572
## mod4  2 137.3337
```

```
# mod and mod2 do not significantly differ, but mod has slighly lower AIC

#check fit of mod

#do a goodness of fit test
1 - pchisq(summary(mod)$deviance,summary(mod)$df.residual)
```

```
## [1] 0.355115
```

```
#the model fits the data well because p> 0.05

#look at the critical level of dispersion by comparing to the degrees of
freedom
qchisq(0.95, df.residual(mod))
```

```
## [1] 82.52873
```

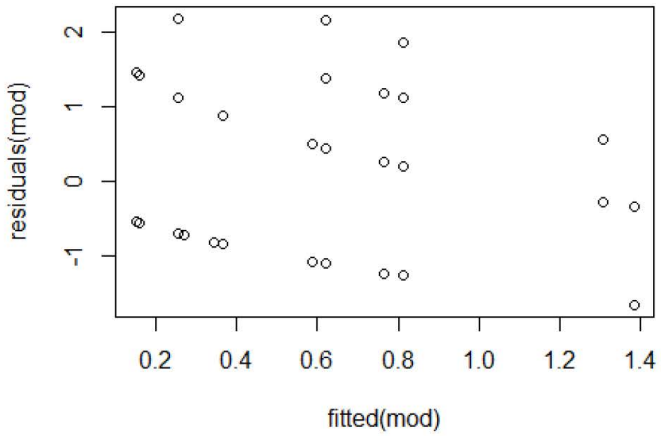
```
#get model deviance
deviance(mod)
```

```
## [1] 66.57188
```

```
#get pearson's chi2
pr <- residuals(mod,"pearson")
sum(pr^2) #if values are lower than critical value, then model fit is good.
```

```
## [1] 72.34497
```

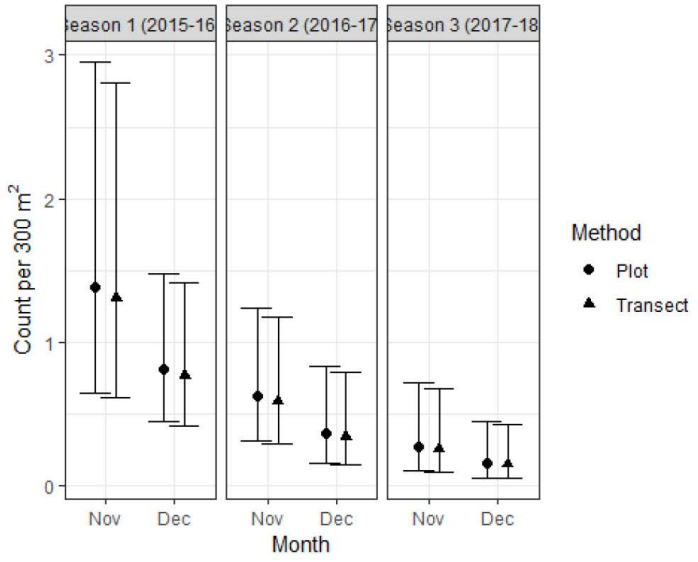
```
plot(fitted(mod), residuals(mod))
```




```
#this model fits the data well, evidence of overdispersion because
deviance>df, but only slightly, and all tests show that not significant.

summary(mod)

##
## Call:
## glm(formula = total ~ Method + Month + Season, family = poisson,
##     data = af)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -1.6642  -0.8532  -0.6386   0.4527   2.1782
##
## Coefficients:
##                Estimate Std. Error z value Pr(>|z|)
## (Intercept)    -0.20957    0.30754  -0.681  0.49559
## MethodTransect -0.05716    0.33820  -0.169  0.86579
## MonthNov        0.53517    0.36676   1.459  0.14452
## Season2        -0.80101    0.40131  -1.996  0.04594 *
## Season3        -1.63384    0.51994  -3.142  0.00168 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for poisson family taken to be 1)
##
##     Null deviance: 79.083  on 67  degrees of freedom
```

```
## Residual deviance: 66.572  on 63  degrees of freedom
## AIC: 130.85
##
## Number of Fisher Scoring iterations: 6

# ------------------------
# predict the mean values
# ------------------------

#make new dataset
nd <- expand.grid(Method = levels(af$Method),
                  Month = levels(af$Month),
                  Season = levels(af$Season))

#print out the means
cbind(nd,
      Mean = predict(mod, newdata=nd, type="response"),
      SE = predict(mod, newdata=nd, type="response", se.fit=T)$se.fit
)

##      Method Month Season      Mean         SE
## 1      Plot   Dec      1 0.8109303 0.24939407
## 2  Transect   Dec      1 0.7658786 0.23957346
## 3      Plot   Nov      1 1.3848503 0.53384827
## 4  Transect   Nov      1 1.3079142 0.50970375
## 5      Plot   Dec      2 0.3640077 0.15322584
## 6  Transect   Dec      2 0.3437851 0.14604169
## 7      Plot   Nov      2 0.6216271 0.21714000
## 8  Transect   Nov      2 0.5870923 0.20780480
## 9      Plot   Dec      3 0.1582770 0.08382553
## 10 Transect   Dec      3 0.1494838 0.07962842
## 11     Plot   Nov      3 0.2702944 0.13387924
## 12 Transect   Nov      3 0.2552781 0.12728086

newY <- predict(mod, newdata = nd, type = "link", se.fit = TRUE)

pd <- data.frame(nd, total = newY)
pd <- mutate(pd, total=exp(total.fit))
pd <- mutate(pd, ucl=exp(total.fit + 1.96*total.se.fit))
pd <- mutate(pd, lcl=exp(total.fit - 1.96*total.se.fit))

# Graph the output

library(ggplot2)

levels(pd$Season) <- c("Season 1 (2015-16)", "Season 2 (2016-17)", "Season 3
(2017-18)")

#reorder the months
pd$Month <- factor(pd$Month, levels=c("Nov", "Dec"))
```

```
ggplot(pd, aes(x = Month, y = total, ymax=ucl, ymin=lcl, shape = Method)) +
  geom_point(position=position_dodge(width=0.6), size = 2) +
  geom_errorbar(position=position_dodge(width=0.6))+
  theme_bw() +
  facet_wrap(~ Season) +
  labs(x = "Month", y = expression ("Count per 300"~m^2))
```




```
# --------------------
# Index of Dispersion
# --------------------

library(dplyr)

# -----------------
# Season 1: Plots
# -----------------

plots1 <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Nov 18 Analysis/PatplotsS1.csv', header = TRUE)

#make sampling session a factor
plots1$Sampling.session <- factor(plots1$Sampling.session)

#put the months in the right order
plots1$Month <- factor(plots1$Month, levels=c("Nov", "Dec","Jan", "Feb",
```

```
"Mar","Apr"))

#make length a numeric
plots1$Length <- as.numeric(as.character(plots1$Length))

#summarise data to get the number of individuals counted per plot group
(100m)
plots1_density100 <- plots1 %>%
  group_by(Month, Sampling.session, Plot) %>%
  summarise(total = sum(Sex != '0'))
plots1_density100$Name <- "Plot_S1_100"
plots1_density100$Transect <- NA
plots1_density100$Method <- "Plot"
plots1_density100$Season <- 1

#summarise data to get the number of individuals counted per sampling session
(300m)
plots1_density300 <- plots1 %>%
  group_by(Month, Sampling.session) %>%
  summarise(total = sum(Sex != '0'))
plots1_density300$Name <- "Plot_S1_300"
plots1_density300$Method <- "Plot"
plots1_density300$Season <- 1


# --------------------
# Season 1: Transects
# --------------------

trans1 <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Nov 18 Analysis/PattransS1.csv', header = TRUE)

#make sampling session a factor
trans1$Sampling.session <- factor(trans1$Sampling.session)

#put the months in the right order
trans1$Month <- factor(trans1$Month, levels=c("Nov", "Dec","Jan", "Feb",
"Mar","Apr"))

#make length a numeric
trans1$Length <- as.numeric(as.character(trans1$Length))

#summarise data to get the number of individuals counted per plot group
(100m)
trans1_density100 <- trans1 %>%
  group_by(Month, Sampling.session, Transect) %>%
  summarise(total = sum(Sex != '0'))
trans1_density100$Name <- "Transect_S1_100"
trans1_density100$Plot <- NA
```

```
trans1_density100$Method <- "Transect"
trans1_density100$Season <- 1

#summarise data to get the number of individuals counted per sampling session
(300m)
trans1_density300 <- trans1 %>%
  group_by(Month, Sampling.session) %>%
  summarise(total = sum(Sex != '0'))
trans1_density300$Name <- "Transect_S1_300"
trans1_density300$Method <- "Transect"
trans1_density300$Season <- 1

# ----------------
#Season 2: Plots
# ----------------

plots2 <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Nov 18 Analysis/PatplotsS2.csv', header = TRUE)

#make sampling session a factor
plots2$Sampling.session <- factor(plots2$Sampling.session)

#put the months in the right order
plots2$Month <- factor(plots2$Month, levels=c("Nov", "Dec","Jan", "Feb",
"Mar","Apr"))

#summarise data to get the number of individuals counted per plot group
(100m)
plots2_density100 <- plots2 %>%
  group_by(Month, Sampling.session, Plot) %>%
  summarise(total = sum(Sex != '0'))
plots2_density100$Name <- "Plot_S2_100"
plots2_density100$Transect <- NA
plots2_density100$Method <- "Plot"
plots2_density100$Season <- 2

#summarise data to get the number of individuals counted per sampling session
(300m)
plots2_density300 <- plots2 %>%
  group_by(Month, Sampling.session) %>%
  summarise(total = sum(Sex != '0'))
plots2_density300$Name <- "Plot_S2_300"
plots2_density300$Method <- "Plot"
plots2_density300$Season <- 2

# --------------------
# Season 2: Transects
# --------------------
```

```
trans2 <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Nov 18 Analysis/PattransS2.csv', header = TRUE)

#make sampling session a factor
trans2$Sampling.session <- factor(trans2$Sampling.session)

#put the months in the right order
trans2$Month <- factor(trans2$Month, levels=c("Nov", "Dec","Jan", "Feb",
"Mar","Apr"))

#make length a numeric
#trans2$Length <- as.numeric(as.character(trans2$Length))

#summarise data to get the number of individuals counted per plot group
(100m)
trans2_density100 <- trans2 %>%
  group_by(Month, Sampling.session, Transect) %>%
  summarise(total = sum(Sex != '0'))
trans2_density100$Name <- "Transect_S2_300"
trans2_density100$Plot <- NA
trans2_density100$Method <- "Transect"
trans2_density100$Season <- 2

#summarise data to get the number of individuals counted per sampling session
(300m)
trans2_density300 <- trans2 %>%
  group_by(Month, Sampling.session) %>%
  summarise(total = sum(Sex != '0'))
trans2_density300$Name <- "Transect_S2_300"
trans2_density300$Method <- "Transect"
trans2_density300$Season <- 2

#----------------
# Season 3: Plots
# ---------------

plots3 <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Nov 18 Analysis/PatplotsS3.csv', header = TRUE)

#make sampling session a factor
plots3$Sampling.session <- factor(plots3$Sampling.session)

#put the months in the right order
plots3$Month <- factor(plots3$Month, levels=c("Nov", "Dec","Jan", "Feb",
"Mar","Apr"))

#summarise data to get the number of individuals counted per plot group
(100m)
plots3_density100 <- plots3 %>%
```

```
  group_by(Month, Sampling.session, Plot) %>%
  summarise(total = sum(Sex != '0'))
plots3_density100$Name <- "Plot_S3_100"
plots3_density100$Transect <- NA
plots3_density100$Method <- "Plot"
plots3_density100$Season <- 3

#summarise data to get the number of individuals counted per sampling session
(300m)
plots3_density300 <- plots3 %>%
  group_by(Month, Sampling.session) %>%
  summarise(total = sum(Sex != '0'))
plots3_density300$Name <- "Plot_S3_300"
plots3_density300$Method <- "Plot"
plots3_density300$Season <- 3

# -------------------
# Season 3: Transects
# -------------------

trans3 <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Nov 18 Analysis/PattransS3.csv', header = TRUE)

#make sampling session a factor
trans3$Sampling.session <- factor(trans3$Sampling.session)

#put the months in the right order
trans3$Month <- factor(trans3$Month, levels=c("Nov", "Dec","Jan", "Feb",
"Mar","Apr"))

#summarise data to get the number of individuals counted per plot group
(100m)
trans3_density100 <- trans3 %>%
  group_by(Month, Sampling.session, Transect) %>%
  summarise(total = sum(Sex != '0'))
trans3_density100$Name <- "Transect_S3_100"
trans3_density100$Plot <- NA
trans3_density100$Method <- "Transect"
trans3_density100$Season <- 3

#summarise data to get the number of individuals counted per sampling session
(300m)
trans3_density300 <- trans3 %>%
  group_by(Month, Sampling.session) %>%
  summarise(total = sum(Sex != '0'))
trans3_density300$Name <- "Transect_S3_300"
trans3_density300$Method <- "Transect"
trans3_density300$Season <- 3
```

```r
# ------------------------------------------
# combine the datasets into a single dataframe ----
# ------------------------------------------

density_100_FULL <- rbind(plots1_density100,
plots2_density100,plots3_density100, trans1_density100, trans2_density100,
trans3_density100)

density_300_FULL <- rbind(plots1_density300,
plots2_density300,plots3_density300, trans1_density300, trans2_density300,
trans3_density300)

# ------------------------------
# SNOWY S2 (first year at Snowy) ----
# ------------------------------

snow2 <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Nov 18 Analysis/SnowyS2.csv', header = TRUE)

#make sampling session a factor
snow2$Sampling.session <- factor(snow2$Sampling.session)

#put the months in the right order
snow2$Month <- factor(snow2$Month, levels=c("Nov", "Dec","Jan", "Feb",
"Mar"))

#summarise data to get the number of individuals counted per plot group
(100m)
snow2_density100 <- snow2 %>%
  group_by(Month, Sampling.session, Transect) %>%
  summarise(total = sum(Sex != '0'))
snow2_density100$Name <- "Snow_S2_100"
snow2_density100$Plot <- NA
snow2_density100$Method <- "SnowyT"
snow2_density100$Season <- 2

# ------------------------------
# SNOWY S3 (second year at Snowy) ----
# ------------------------------

snow3 <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Nov 18 Analysis/SnowyS3.csv', header = TRUE)

#make sampling session a factor
snow3$Sampling.session <- factor(snow3$Sampling.session)

#put the months in the right order
snow3$Month <- factor(snow3$Month, levels=c("Nov", "Dec","Jan", "Feb",
"Mar"))
```

```
#summarise data to get the number of individuals counted per plot group
(100m)
snow3_density100 <- snow3 %>%
  group_by(Month, Sampling.session, Transect) %>%
  summarise(total = sum(Sex != '0'))
snow3_density100$Name <- "Snow_S3_100"
snow3_density100$Plot <- NA
snow3_density100$Method <- "SnowyT"
snow3_density100$Season <- 3

# -----------------------------
# Step 1: Generate variability estimates for each transects, in each month,
for each sampling stretegy, for each season ----
# -----------------------------

#join the datasets together to make a new one
var_data <- rbind (density_100_FULL, snow2_density100, snow3_density100)

#put plot ID and transect ID into the same column
var_data$Transect <- as.factor(var_data$Transect)
var_data$ID <- ifelse(is.na(var_data$Transect),var_data$Plot,
var_data$Transect)

var_data$Month <- as.factor(as.character(var_data$Month))
var_data$Name <- as.factor(as.character(var_data$Name))
var_data$Method <- as.factor(as.character(var_data$Method))
var_data <- var_data[var_data$Month != 'Apr',]
var_data <- droplevels(var_data)
var_data$Season <- as.factor(as.numeric(var_data$Season))

# -----------------------------
# generate the mean and standard deviation for each of the groups in the
dataframe
# -----------------------------

library(plyr)

## ------------------------------------------------------------------------------

## You have loaded plyr after dplyr - this is likely to cause problems.
## If you need functions from both plyr and dplyr, please load plyr first,
then dplyr:
## library(plyr); library(dplyr)

## ------------------------------------------------------------------------------

##
## Attaching package: 'plyr'
```

```
## The following objects are masked from 'package:dplyr':
##
##     arrange, count, desc, failwith, id, mutate, rename, summarise,
##     summarize

library(dplyr)
library(reshape2)

var_data_output<- ddply(var_data, c("Method", "Name", "Season", "Month",
"ID"), summarise,
                        mean = mean(total), sd = sd(total),
                        sem = sd(total)/sqrt(length(total)),
                        var = var(total),
                        D = var(total)/mean(total))
var_data_output$Log_D <- log(var_data_output$D)

# drop season 1 because Snowy River data is missing
var_data_output <- var_data_output[var_data_output$Season != '1',]
var_data_output <- droplevels(var_data_output)

# --------------
# write model
# --------------

library(lme4)
library(lmerTest)

# model 1

mod1 <- lmer(D ~ Method + (1|ID), data = var_data_output, REML = T)

# look at output
summary(mod1)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: D ~ Method + (1 | ID)
##    Data: var_data_output
##
## REML criterion at convergence: 261.8
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.4407 -0.6653 -0.2377  0.4203  4.5812
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  ID       (Intercept) 0.03458  0.1859
##  Residual             0.83162  0.9119
## Number of obs: 97, groups:  ID, 5
```

```
##
## Fixed effects:
##                Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)     1.61182    0.19603 11.67545   8.222  3.4e-06 ***
## MethodSnowyT   -0.38993    0.22659 93.90437  -1.721   0.0886 .
## MethodTransect -0.07402    0.23546 91.56199  -0.314   0.7540
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) MthdST
## MethodSnwyT -0.670
## MethdTrnsct -0.601  0.520
```

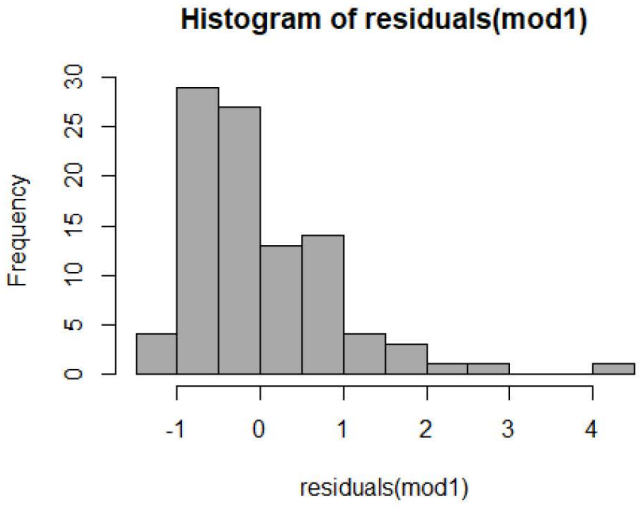
```
anova(mod1)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
##        Sum Sq Mean Sq NumDF  DenDF F value Pr(>F)
## Method 2.8457  1.4229     2 92.272   1.711 0.1864
```

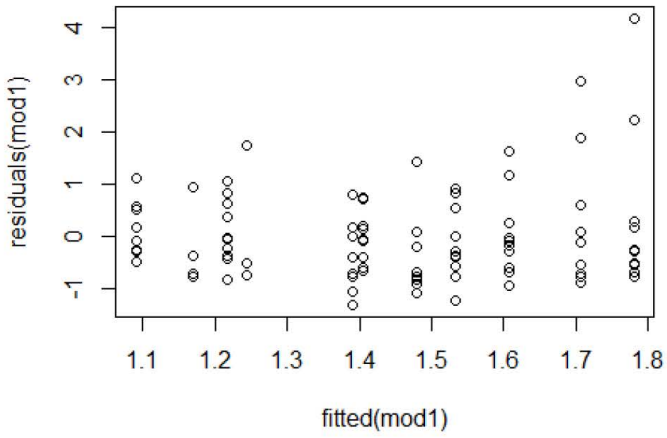
```
difflsmeans(mod1, test.effs = "Method")
```

```
## Least Squares Means table:
##
##                                 Estimate Std. Error   df t value     lower
## MethodPlot - MethodSnowyT      0.389930   0.226586 93.9  1.7209 -0.059967
## MethodPlot - MethodTransect    0.074019   0.235460 91.6  0.3144 -0.393654
## MethodSnowyT - MethodTransect -0.315911   0.226586 93.9 -1.3942 -0.765808
##                                    upper Pr(>|t|)
## MethodPlot - MethodSnowyT      0.839827  0.08856 .
## MethodPlot - MethodTransect    0.541692  0.75396
## MethodSnowyT - MethodTransect  0.133986  0.16654
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##   Confidence level: 95%
##   Degrees of freedom method: Satterthwaite
```

```
#check for normal residuals
hist(residuals(mod1),col="darkgray")
```




```
#check for homoscedastic residulas
plot(fitted(mod1),residuals(mod1))
```



```r
difflsmeans(mod1, test.effs = "Method")

## Least Squares Means table:
##
##                                 Estimate Std. Error   df t value     lower
## MethodPlot - MethodSnowyT      0.389930   0.226586 93.9  1.7209 -0.059967
## MethodPlot - MethodTransect    0.074019   0.235460 91.6  0.3144 -0.393654
## MethodSnowyT - MethodTransect -0.315911   0.226586 93.9 -1.3942 -0.765808
##                                    upper Pr(>|t|)
## MethodPlot - MethodSnowyT      0.839827  0.08856 .
## MethodPlot - MethodTransect    0.541692  0.75396
## MethodSnowyT - MethodTransect  0.133986  0.16654
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##   Confidence level: 95%
##   Degrees of freedom method: Satterthwaite

# ------------------------------------
# try re-running the analysis after removing the outliers and see what the
results are
# ------------------------------------

# remove outliers from data
dat_no_out <- var_data_output[var_data_output$D < 3,]

# model 2
mod2 <- lmer(D ~ Method + Month + (1|Season) + (1|ID), data = dat_no_out,
REML = T)

## singular fit

# look at output
summary(mod2)

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: D ~ Method + Month + (1 | Season) + (1 | ID)
##    Data: dat_no_out
##
## REML criterion at convergence: 175.9
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.9662 -0.6723 -0.2041  0.5088  2.5745
##
## Random effects:
##  Groups   Name        Variance  Std.Dev.
##  ID       (Intercept) 8.069e-17 8.983e-09
##  Season   (Intercept) 0.000e+00 0.000e+00
##  Residual             3.738e-01 6.114e-01
```

```
## Number of obs: 91, groups:  ID, 5; Season, 2
##
## Fixed effects:
##                Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)     1.25066    0.17623 84.00000   7.097 3.76e-10 ***
## MethodSnowyT   -0.12270    0.15586 84.00000  -0.787    0.433
## MethodTransect  0.05319    0.16498 84.00000   0.322    0.748
## MonthFeb       -0.16786    0.20398 84.00000  -0.823    0.413
## MonthJan        0.20355    0.20685 84.00000   0.984    0.328
## MonthMar        0.17755    0.20117 84.00000   0.883    0.380
## MonthNov        0.07528    0.20117 84.00000   0.374    0.709
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) MthdST MthdTr MnthFb MnthJn MnthMr
## MethodSnwyT -0.512
## MethdTrnsct -0.497  0.539
## MonthFeb    -0.578 -0.018  0.022
## MonthJan    -0.576  0.000  0.022  0.493
## MonthMar    -0.601  0.024  0.023  0.506  0.499
## MonthNov    -0.601  0.024  0.023  0.506  0.499  0.514
## convergence code: 0
## singular fit
```

```
anova(mod2)
```

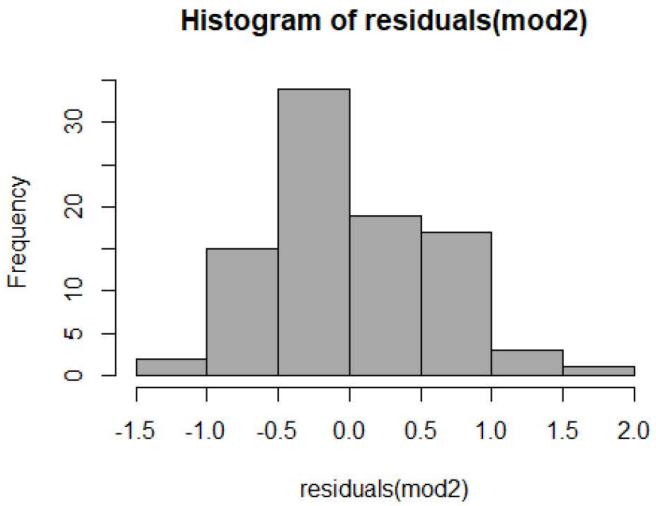
```
## Type III Analysis of Variance Table with Satterthwaite's method
##         Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## Method 0.52521 0.26260     2    84  0.7025 0.4982
## Month  1.61375 0.40344     4    84  1.0793 0.3720
```

```
difflsmeans(mod2, test.effs = "Method")
```

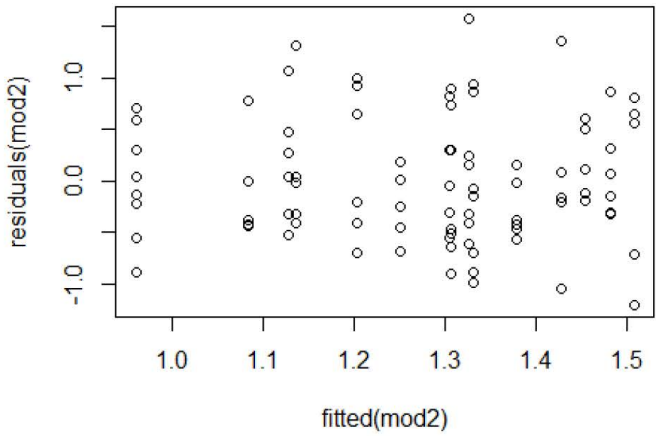
```
## Least Squares Means table:
##
##                                Estimate Std. Error df t value     lower
## MethodPlot - MethodSnowyT      0.122700   0.155856 84  0.7873 -0.187235
## MethodPlot - MethodTransect   -0.053187   0.164975 84 -0.3224 -0.381259
## MethodSnowyT - MethodTransect -0.175888   0.154282 84 -1.1400 -0.482693
## MonthDec - MonthFeb            0.167856   0.203979 84  0.8229 -0.237779
## MonthDec - MonthJan           -0.203551   0.206846 84 -0.9841 -0.614888
## MonthDec - MonthMar           -0.177546   0.201171 84 -0.8826 -0.577595
## MonthDec - MonthNov           -0.075282   0.201171 84 -0.3742 -0.475332
## MonthFeb - MonthJan           -0.371408   0.206819 84 -1.7958 -0.782690
## MonthFeb - MonthMar           -0.345402   0.201347 84 -1.7155 -0.745803
## MonthFeb - MonthNov           -0.243138   0.201347 84 -1.2076 -0.643539
## MonthJan - MonthMar            0.026006   0.204194 84  0.1274 -0.380056
## MonthJan - MonthNov            0.128269   0.204194 84  0.6282 -0.277792
## MonthMar - MonthNov            0.102264   0.198364 84  0.5155 -0.292204
##                                   upper Pr(>|t|)
```

```
## MethodPlot - MethodSnowyT      0.432636  0.43334
## MethodPlot - MethodTransect    0.274885  0.74796
## MethodSnowyT - MethodTransect  0.130918  0.25751
## MonthDec - MonthFeb            0.573491  0.41289
## MonthDec - MonthJan            0.207785  0.32791
## MonthDec - MonthMar            0.222504  0.37999
## MonthDec - MonthNov            0.324768  0.70918
## MonthFeb - MonthJan            0.039875  0.07612 .
## MonthFeb - MonthMar            0.055000  0.08995 .
## MonthFeb - MonthNov            0.157263  0.23061
## MonthJan - MonthMar            0.432068  0.89896
## MonthJan - MonthNov            0.534331  0.53159
## MonthMar - MonthNov            0.496731  0.60753
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##   Confidence level: 95%
##   Degrees of freedom method: Satterthwaite
```

```
#check for normal residuals
hist(residuals(mod2),col="darkgray")
```



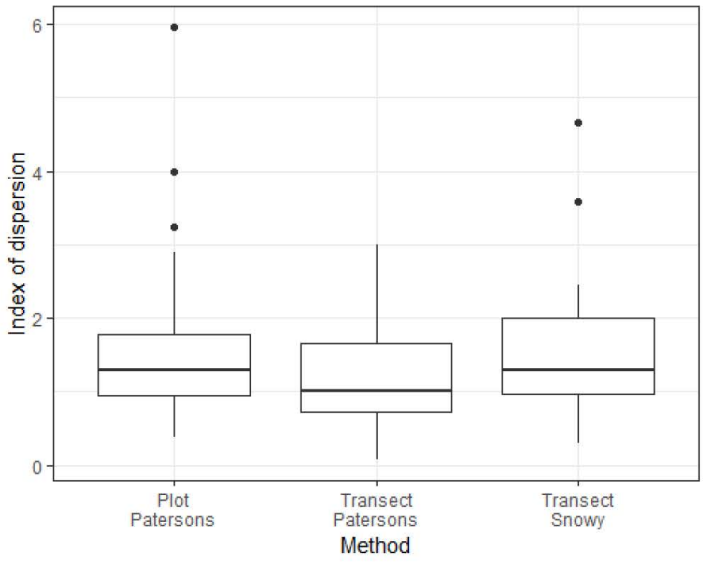

```
#check for homoscedastic residulas
plot(fitted(mod2),residuals(mod2))
```




```
# no difference in findings from results, stick with model1 that contains all
the data points

# -----------------
# post-hoc analysis
# -----------------

library(multcomp)

## Loading required package: mvtnorm

## Loading required package: survival

## Loading required package: TH.data

##
## Attaching package: 'TH.data'

## The following object is masked from 'package:MASS':
##
##     geyser

summary(glht(mod2, linfct = mcp(Month = "Tukey")))

##
##   Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
```

```
##
##
## Fit: lmer(formula = D ~ Method + Month + (1 | Season) + (1 | ID),
##     data = dat_no_out, REML = T)
##
## Linear Hypotheses:
##                 Estimate Std. Error z value Pr(>|z|)
## Feb - Dec == 0 -0.16786    0.20398  -0.823    0.924
## Jan - Dec == 0  0.20355    0.20685   0.984    0.863
## Mar - Dec == 0  0.17755    0.20117   0.883    0.903
## Nov - Dec == 0  0.07528    0.20117   0.374    0.996
## Jan - Feb == 0  0.37141    0.20682   1.796    0.376
## Mar - Feb == 0  0.34540    0.20135   1.715    0.424
## Nov - Feb == 0  0.24314    0.20135   1.208    0.747
## Mar - Jan == 0 -0.02601    0.20419  -0.127    1.000
## Nov - Jan == 0 -0.12827    0.20419  -0.628    0.971
## Nov - Mar == 0 -0.10226    0.19836  -0.516    0.986
## (Adjusted p values reported -- single-step method)
```

```
ggplot(var_data_output, aes(y = D, x= Method)) +
  geom_boxplot() +
  #facet_wrap(~ Month) +
  theme_bw()+
  ylab("Index of dispersion") +
  #geom_text(data = label.df  label = "*") +
  scale_x_discrete(labels =
c('Plot\nPatersons','Transect\nPatersons','Transect\nSnowy'))
```



```
# ------------------------------------------------
# simulation for transect monitoring power analysis
# ------------------------------------------------

# open packages
library(lme4)

# -------------------
# Select model to use
# -------------------

#
# Patersons Terrace | Large females | November + December counts
#

#set.seed(20190805)

# read in data
pattAF <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Power_analysis_transects/Power_Sept_04/Patersons_AF_NovDec_dat.csv
')

# model selection
mod <- glmer(count ~ Year_t + (1|Transect) + (1|Visit) + (1|Individual),
family = poisson, data = pattAF)

## singular fit

mod1 <- glmer(count ~ Year_t + (1|Transect) + (1|Visit), family = poisson,
data = pattAF)

AIC(mod, mod1)

##      df      AIC
## mod   5 113.0695
## mod1  4 111.0695

anova(mod, mod1)

## Data: pattAF
## Models:
## mod1: count ~ Year_t + (1 | Transect) + (1 | Visit)
## mod: count ~ Year_t + (1 | Transect) + (1 | Visit) + (1 | Individual)
##      Df    AIC    BIC  logLik deviance Chisq Chi Df Pr(>Chisq)
## mod1  4 111.07 121.57 -51.535   103.07
## mod   5 113.07 126.19 -51.535   103.07     0      1          1

# No sig diff in models, but mod1 has lower AIC

#
```

```
# Patersons Terrace | Total population | February counts
#

# set.seed(20190805)

# read in data
pat <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Power_analysis_transects/Power_Sept_04/Patersons_Tp_Feb_dat.csv')

# model selection
mod <- glmer(count ~ Year_t + (1|Transect) + (1|Visit) + (1|Individual),
family = poisson, data = pat)

## singular fit

mod1 <- glmer(count ~ Year_t + (1|Transect) + (1|Visit), family = poisson,
data = pat)

## singular fit

AIC(mod, mod1)

##      df      AIC
## mod   5 230.7726
## mod1  4 235.3944

anova(mod, mod1)

## Data: pat
## Models:
## mod1: count ~ Year_t + (1 | Transect) + (1 | Visit)
## mod: count ~ Year_t + (1 | Transect) + (1 | Visit) + (1 | Individual)
##      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## mod1  4 235.39 243.35 -113.70   227.39
## mod   5 230.77 240.72 -110.39   220.77 6.6218      1    0.01007 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# mod is sig diff to mod1, + lower AIC

#
# Snowy River | Adult Females | November and December counts
#

# set.seed(20190805)

# read in data
snowAF <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Power_analysis_transects/Power_Sept_04/Snowy_AF_NovDec_dat.csv')

# model selection
```

```
mod <- glmer(count ~ Year_t + (1|Transect) + (1|Visit) + (1|Individual),
family = poisson, data = snowAF)

## singular fit

mod1 <- glmer(count ~ Year_t + (1|Transect) + (1|Visit), family = poisson,
data = snowAF)

## singular fit

AIC(mod, mod1)

##      df      AIC
## mod   5 73.91539
## mod1  4 75.09072

anova(mod, mod1)

## Data: snowAF
## Models:
## mod1: count ~ Year_t + (1 | Transect) + (1 | Visit)
## mod: count ~ Year_t + (1 | Transect) + (1 | Visit) + (1 | Individual)
##      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## mod1  4 75.091 83.663 -33.545   67.091
## mod   5 73.915 84.631 -31.958   63.915 3.1753      1    0.07476 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# No sig diff in models, but mod has lower AIC

#
# Snowy River | Total population | February counts
#

#set.seed(20190805)

# read in data
snow <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Power_analysis_transects/Power_Sept_04/Snowy_Tp_Feb_dat.csv')

# model selection
mod <- glmer(count ~ Year_t + (1|Transect) + (1|Visit) + (1|Individual),
family = poisson, data = snow)

## singular fit

mod1 <- glmer(count ~ Year_t + (1|Transect) + (1|Visit), family = poisson,
data = snow)

AIC(mod, mod1)
```

```
##      df     AIC
## mod   5 111.257
## mod1  4 109.257

anova(mod, mod1)

## Data: snow
## Models:
## mod1: count ~ Year_t + (1 | Transect) + (1 | Visit)
## mod: count ~ Year_t + (1 | Transect) + (1 | Visit) + (1 | Individual)
##      Df    AIC    BIC  logLik deviance Chisq Chi Df Pr(>Chisq)
## mod1  4 109.26 114.86 -50.629   101.26
## mod   5 111.26 118.26 -50.629   101.26     0      1     0.9998

# No sig diff in models, but mod1 has lower AIC

# -------------------------
# prepare to run simulation
# -------------------------

# number of simulations to run
nsim <- 1000

# number of transects
transects <- seq(3, 40, by=1)

#number of visits
visits <- seq(2, 25, by=1)

# storing results
powersummary <- data.frame(matrix(NA, ncol = 25, nrow = 40))

newdata <- data.frame()

pval <- list()

# ---------------
# run simulations
# ---------------

#
# Model that does not include an 'individual' parameter
#

#for (t in transects){
#  for (v in visits) {

#    newdata <- expand.grid(Visit = as.factor(1:v),
#                           Transect = as.factor(1:t),
```

```
#                           Year_t = c(0,1))          #Year_t = c(0,1,2)) for
Patersons Terrace data

    #create 'nsim' replicates of results using the new data frame
#    simdat <- simulate(mod1, nsim=nsim, newdata=newdata, re.form=~0,
allow.new.levels = TRUE, family=poisson())

#    for(j in 1:nsim){

      #print the number of the simulation
#      print(j)

      #using a the results of simulation j, create a data frame with
response, transect, visits and         year variables
#      sdat <- data.frame(response=simdat[,j], newdata)

      # if all the responses are 0, return p value of 1
#      if (sum(sdat$response == 0) == length(sdat$response)) {
#        pval[j] <- 1

        #otherwise, fit the model to the new data
#      } else {
#        smod <- try(glmer(response ~ Year_t + (1|Transect) +
#                            (1|Visit), family = poisson, data = sdat))

        #if the model returns an error (cannot be fit), the return a p vlaue
of 1
#         if (class(smod)[1] == "try-error"){
#          pval[j] <- 1

          #otherwise, return the p-value for the fixed effect "Year"
#        } else {
#          pval[j] <- coef(summary(smod))["Year_t","Pr(>|z|)"]
#       }
#      }}
    #calulate the power for each number of transects and visits
#    powersummary[t,v] <- sum(pval < 0.05)/nsim

#  }}

#
# Model that does include an 'individual' parameter
#

#for (t in transects){
#  for (v in visits) {

#    newdata <- expand.grid(Visit = as.factor(1:v),
#                           Transect = as.factor(1:t),
```

```
#                           Year_t = c(0,1))          #Year_t = c(0,1,2)) for
Patersons Terrace data
#    newdata$Individual <- as.factor(1:nrow(newdata))

    #create 'nsim' replicates of results using the new data frame
#    simdat <- simulate(mod1, nsim=nsim, newdata=newdata, re.form=~0,
allow.new.levels = TRUE, family=poisson())

#    for(j in 1:nsim){

      #print the number of the simulation
#      print(j)

      #using a the results of simulation j, create a data frame with
response, transect, visits and         year variables
#      sdat <- data.frame(response=simdat[,j], newdata)

      # if all the responses are 0, return p value of 1
#      if (sum(sdat$response == 0) == length(sdat$response)) {
#        pval[j] <- 1

        #otherwise, fit the model to the new data
#      } else {
#        smod <- try(glmer(response ~ Year_t + (1|Transect) +
#                            (1|Visit) + (1|Individual), family = poisson,
data = sdat))

        #if the model returns an error (cannot be fit), the return a p vlaue
of 1
#        if (class(smod)[1] == "try-error"){
#          pval[j] <- 1

          #otherwise, return the p-value for the fixed effect "Year"
#        } else {
#          pval[j] <- coef(summary(smod))["Year_t","Pr(>|z|)"]
#        }
#      }}
    #calulate the power for each number of transects and visits
#    powersummary[t,v] <- sum(pval < 0.05)/nsim

#  }}

# ---------------------------------------
# Logistic regression to determine the detectability along transects at
various scales
# ---------------------------------------

# ------------------------------
# Prepare data at 100m resolution
```

```
# ------------------------------

library(dplyr)

#read in the data
dat<-read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Combined data filtered.csv',na.strings = "")

# make the NAs in the sex column a '0'
dat$Sex[is.na (dat$Sex)] <- 0

#create new column with 0 or 1 or represent when a grasshopper is present
dat$Present <- (dat$Sex != "NA")
dat$Present <- as.integer(as.logical(dat$Present))

# drop columns that aren't needed
dat <- dat %>%

select_("Present","Sex","Visit_All_time","Location","Transect","Season","Mont
h","Ground","Baro", "Wind", "Sky", "Standard_time")

#add time squared variable
dat$time_sq <- dat$Standard_time*dat$Standard_time

#make column with standardised ground temperature
dat$Ground <- as.numeric(as.character(dat$Ground))
dat$Ground_standard <- (dat$Ground - mean(dat$Ground, na.rm = T)) /
sd(dat$Ground, na.rm = T)
dat$temp_sq <- dat$Ground_standard*dat$Ground_standard

#rename the transects so that snowy and patersons have different IDs
dat$TransectID <- paste(dat$Location, dat$Transect,sep="-")

#make the new column a factor rather than a character
dat$TransectID <- as.factor(dat$TransectID)

#rescale season so thats its on a better scale
dat$SeasonID <- dat$Season - 2015

#subset for unique rows of info
library(data.table)

##
## Attaching package: 'data.table'

## The following objects are masked from 'package:reshape2':
##
##     dcast, melt
```

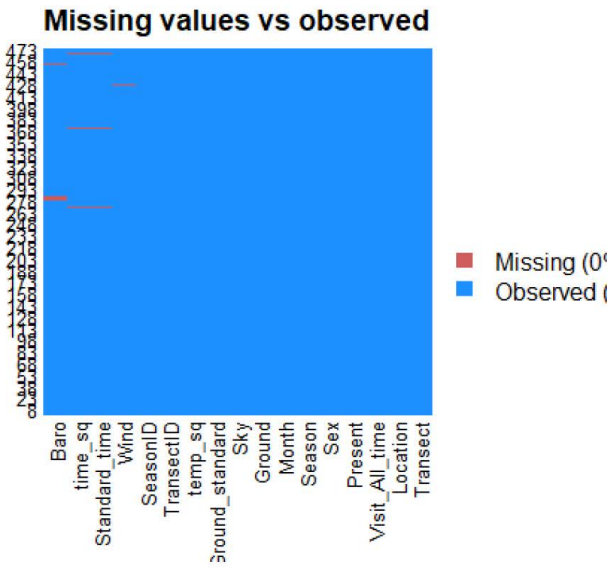
```
## The following objects are masked from 'package:dplyr':
##
##     between, first, last
```

```
dt <- data.table(dat)
dt1<-dt[,.SD[which.max(Present)],by=list(Transect, Location, Visit_All_time)]

#remove April data
dt1<-dt1[(dt1$Month != "Apr"),]

#drop rows where temp and sky contain NA
dt1 <- dt1[dt1$Ground != 'NA',]
dt1 <- dt1[dt1$Sky != 'NA',]
dt1 <- droplevels(dt1)

#check for missing values
library(Amelia)
```

```
## Loading required package: Rcpp
```

```
## ##
## ## Amelia II: Multiple Imputation
## ## (Version 1.7.5, built: 2018-05-07)
## ## Copyright (C) 2005-2020 James Honaker, Gary King and Matthew Blackwell
## ## Refer to http://gking.harvard.edu/amelia/ for more information
## ##
```

```
missmap(dt1, main = "Missing values vs observed")
```




```
# ----------------------------
# Logistic Regression Analysis
# ----------------------------

# read in data
dat <- read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Full popn logistic regression data 100m.csv')

dat$SeasonID <- as.factor(as.integer(dat$SeasonID))

library(plyr)
dat$Location <- revalue(dat$Location, c("Pattersons"="Patersons Terrace",
"Snowy"="Snowy River"))

#variables of interest: time, time_sq, location, transect ID (random!),
sky/cloud cover, temperature, temperature_sq, season (random?)
# note that there is no temperature for the November data

#Explore the dataset

#run logistic regression
library(lme4)
library(lmerTest)

#Exclude temperature from the anaylsis because no data for November in the
first year
```

```r
#full model
mod1 <- glmer(Present ~ Location + Month + Sky + Ground_standard +
(1|SeasonID) + (1|TransectID) ,  family = "binomial", data = dat)

#check for overdispersion
E1 <- residuals(mod1)
p1 <- length(fixef(mod1)) + 1
(overdisp1 <- sum(E1^2) /(nrow(dat) - p1))
```

```
## [1] 0.8755214
```

```r
#value has to be less than 1 (which it is)

#basic informative model
mod2 <- glmer(Present ~ Location + Month + Sky + (1|SeasonID) +
(1|TransectID),  family = "binomial", data = dat)

#check for overdispersion
E1 <- residuals(mod2)
p1 <- length(fixef(mod2)) + 1
(overdisp1 <- sum(E1^2) /(nrow(dat) - p1))
```

```
## [1] 0.8789431
```

```r
#value has to be less than 1 (which it is)

mod3 <- glmer(Present ~ Location + Month + (1|SeasonID) + (1|TransectID),
family = "binomial", data = dat)

mod4 <- glmer(Present ~ Location + Sky + (1|SeasonID) + (1|TransectID),
family = "binomial", data = dat)

anova(mod4, mod2, test = "Chisq")
```

```
## Data: dat
## Models:
## mod4: Present ~ Location + Sky + (1 | SeasonID) + (1 | TransectID)
## mod2: Present ~ Location + Month + Sky + (1 | SeasonID) + (1 | TransectID)
##      Df   AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## mod4  7 469.7 498.82 -227.85    455.7
## mod2 11 451.3 497.05 -214.65    429.3 26.402      4  2.625e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
AIC(mod1, mod2, mod3, mod4)
```

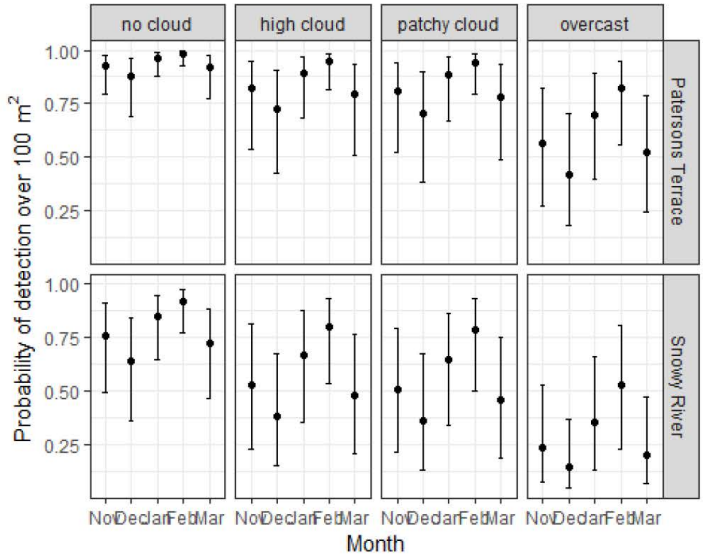
```
##      df      AIC
## mod1 12 450.8770
## mod2 11 451.2989
```

```
## mod3  8 491.4495
## mod4  7 469.7014

#model 2 comes out on top.

summary(mod2)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: binomial  ( logit )
## Formula:
## Present ~ Location + Month + Sky + (1 | SeasonID) + (1 | TransectID)
##    Data: dat
##
##      AIC      BIC   logLik deviance df.resid
##    451.3    497.0   -214.6    429.3      462
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -5.3028 -0.4514  0.2685  0.5314  1.8490
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  TransectID (Intercept) 0.2789   0.5281
##  SeasonID   (Intercept) 0.4597   0.6780
## Number of obs: 473, groups:  TransectID, 8; SeasonID, 3
##
## Fixed effects:
##                     Estimate Std. Error z value Pr(>|z|)
## (Intercept)           0.9698     0.6483   1.496  0.13473
## LocationSnowy River  -1.4340     0.4841  -2.962  0.00305 **
## MonthFeb              1.8682     0.4207   4.441 8.97e-06 ***
## MonthJan              1.1614     0.3803   3.054  0.00226 **
## MonthMar              0.3942     0.3547   1.111  0.26640
## MonthNov              0.5840     0.3921   1.489  0.13638
## Skyno cloud           1.0382     0.4018   2.584  0.00976 **
## Skyovercast          -1.2829     0.4703  -2.728  0.00638 **
## Skypatchy cloud      -0.0928     0.5043  -0.184  0.85402
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) LctnSR MnthFb MnthJn MnthMr MnthNv Skyncl Skyvrc
## LctnSnwyRvr -0.432
## MonthFeb    -0.222 -0.053
## MonthJan    -0.175 -0.043  0.389
## MonthMar    -0.200  0.005  0.382  0.474
## MonthNov    -0.125 -0.012  0.373  0.433  0.454
## Skyno cloud -0.404 -0.006  0.091 -0.135 -0.201 -0.178
```

```
## Skyovercast -0.450  0.129  0.057 -0.056 -0.045 -0.137  0.646
## Skyptchycld -0.323  0.017 -0.045 -0.153 -0.108 -0.179  0.624  0.529

# --------------
# Plot the model
# --------------

#note: make sure the order of the months is alphabetical as it is specified
in the model.
NewData <- expand.grid(Location = levels(dat$Location),
                       Month = levels(dat$Month),
                       Sky = levels(dat$Sky),
                       SeasonID = c(0:20),
                       TransectID = levels(dat$TransectID))

X <- model.matrix(~ Location + Month + Sky,
                  data = NewData)

NewData$eta         <- X %*% fixef(mod2)
NewData$SuccessPred <- exp(NewData$eta) / (1 + exp(NewData$eta))
head(NewData,15)

##             Location Month        Sky SeasonID   TransectID         eta
## 1  Patersons Terrace   Dec high cloud        0 Pattersons-A  0.96975334
## 2        Snowy River   Dec high cloud        0 Pattersons-A -0.46419454
## 3  Patersons Terrace   Feb high cloud        0 Pattersons-A  2.83797978
## 4        Snowy River   Feb high cloud        0 Pattersons-A  1.40403190
## 5  Patersons Terrace   Jan high cloud        0 Pattersons-A  2.13115256
## 6        Snowy River   Jan high cloud        0 Pattersons-A  0.69720468
## 7  Patersons Terrace   Mar high cloud        0 Pattersons-A  1.36399675
## 8        Snowy River   Mar high cloud        0 Pattersons-A -0.06995113
## 9  Patersons Terrace   Nov high cloud        0 Pattersons-A  1.55380804
## 10       Snowy River   Nov high cloud        0 Pattersons-A  0.11986016
## 11 Patersons Terrace   Dec   no cloud        0 Pattersons-A  2.00798273
## 12       Snowy River   Dec   no cloud        0 Pattersons-A  0.57403485
## 13 Patersons Terrace   Feb   no cloud        0 Pattersons-A  3.87620916
## 14       Snowy River   Feb   no cloud        0 Pattersons-A  2.44226129
## 15 Patersons Terrace   Jan   no cloud        0 Pattersons-A  3.16938195
##    SuccessPred
## 1    0.7250703
## 2    0.3859912
## 3    0.9446940
## 4    0.8028229
## 5    0.8938944
## 6    0.6675677
## 7    0.7964085
## 8    0.4825193
## 9    0.8254631
## 10   0.5299292
## 11   0.8816327
```

```
## 12   0.6396937
## 13   0.9796917
## 14   0.9199937
## 15   0.9596657

#Get the standard errors
NewData$VarPred <- diag(X %*% vcov(mod2) %*% t(X))
NewData$sePred  <- sqrt(NewData$VarPred)

NewData$seLow  <- exp(NewData$eta - 1.96 * NewData$sePred) / (1 +
exp(NewData$eta - 1.96 * NewData$sePred))
NewData$seHigh <- exp(NewData$eta + 1.96 * NewData$sePred) / (1 +
exp(NewData$eta + 1.96 * NewData$sePred))
head(NewData, 10)

##             Location Month        Sky SeasonID   TransectID         eta
## 1  Patersons Terrace   Dec high cloud        0 Pattersons-A  0.96975334
## 2        Snowy River   Dec high cloud        0 Pattersons-A -0.46419454
## 3  Patersons Terrace   Feb high cloud        0 Pattersons-A  2.83797978
## 4        Snowy River   Feb high cloud        0 Pattersons-A  1.40403190
## 5  Patersons Terrace   Jan high cloud        0 Pattersons-A  2.13115256
## 6        Snowy River   Jan high cloud        0 Pattersons-A  0.69720468
## 7  Patersons Terrace   Mar high cloud        0 Pattersons-A  1.36399675
## 8        Snowy River   Mar high cloud        0 Pattersons-A -0.06995113
## 9  Patersons Terrace   Nov high cloud        0 Pattersons-A  1.55380804
## 10       Snowy River   Nov high cloud        0 Pattersons-A  0.11986016
##    SuccessPred   VarPred    sePred     seLow    seHigh
## 1    0.7250703 0.4203625 0.6483537 0.4253082 0.9038302
## 2    0.3859912 0.3832245 0.6190513 0.1574222 0.6789926
## 3    0.9446940 0.4762720 0.6901246 0.8153771 0.9850889
## 4    0.8028229 0.4176658 0.6462707 0.5342815 0.9352768
## 5    0.8938944 0.4786053 0.6918130 0.6846445 0.9703187
## 6    0.6675677 0.4254952 0.6523000 0.3586328 0.8782234
## 7    0.7964085 0.4543568 0.6740600 0.5107082 0.9361449
## 8    0.4825193 0.4189149 0.6472363 0.2077512 0.7682814
## 9    0.8254631 0.5107601 0.7146748 0.5381869 0.9504791
## 10   0.5299292 0.4691628 0.6849546 0.2274703 0.8118951

#reorder the levels so that they plot correctly
NewData$Month2 <- factor(NewData$Month, levels = c("Nov", "Dec", "Jan",
"Feb", "Mar"))
NewData$Sky2 <- factor(NewData$Sky, levels = c("no cloud", "high cloud",
"patchy cloud", "overcast"))

dat100 <- NewData

library(ggplot2)

ggplot(data = NewData, aes(Month2, SuccessPred)) +
  geom_point() + #plot the points
```

```
  ylab("Probability detection (100m transect)") +
  theme_bw() +
  geom_errorbar(aes(ymin=seLow, ymax=seHigh), width = 0.2) +
  facet_grid(Location ~ Sky2) +
  labs(x = "Month", y = (expression("Probability of detection over 100
"~m^2)))
```




```
# -------------
# post-hoc test
# -------------

library(multcompView)
library(lsmeans)

## Loading required package: emmeans

##
## Attaching package: 'emmeans'

## The following object is masked from 'package:multcomp':
##
##     cld

## The 'lsmeans' package is now basically a front end for 'emmeans'.
## Users are encouraged to switch the rest of the way.
## See help('transition') for more information, including how to
## convert old 'lsmeans' objects and scripts to work with 'emmeans'.
```

```
marginal <- lsmeans(mod2, ~Location + Month + Sky, adjust = "tukey")

(post_hoc_100 <- cld(marginal,
                     alpha = 0.05,
                     Letters = letters,
                     adjust = "tukey"))
```

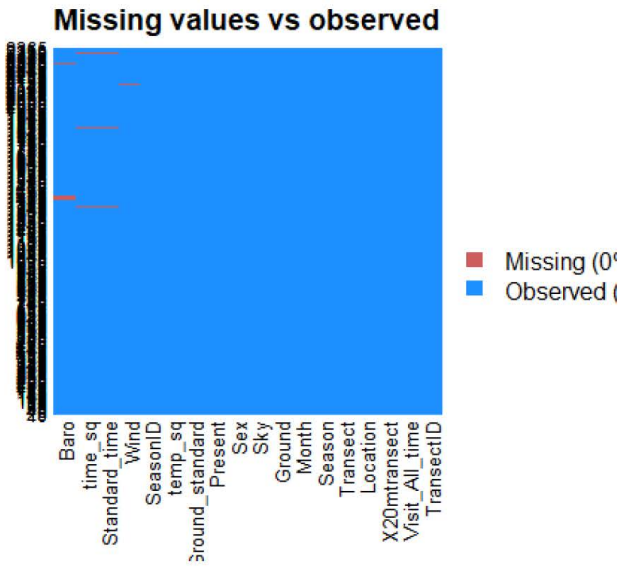
```
##  Location          Month Sky           lsmean    SE  df asymp.LCL
##  Snowy River       Dec   overcast     -1.7471 0.623 Inf   -3.7541
##  Snowy River       Mar   overcast     -1.3528 0.640 Inf   -3.4123
##  Snowy River       Nov   overcast     -1.1630 0.651 Inf   -3.2595
##  Snowy River       Jan   overcast     -0.5857 0.641 Inf   -2.6490
##  Snowy River       Dec   patchy cloud -0.5570 0.659 Inf   -2.6787
##  Snowy River       Dec   high cloud   -0.4642 0.619 Inf   -2.4576
##  Patersons Terrace Dec   overcast     -0.3131 0.606 Inf   -2.2636
##  Snowy River       Mar   patchy cloud -0.1627 0.657 Inf   -2.2769
##  Snowy River       Mar   high cloud   -0.0700 0.647 Inf   -2.1541
##  Snowy River       Nov   patchy cloud  0.0271 0.670 Inf   -2.1316
##  Patersons Terrace Mar   overcast      0.0811 0.621 Inf   -1.9189
##  Snowy River       Nov   high cloud    0.1199 0.685 Inf   -2.0858
##  Snowy River       Feb   overcast      0.1211 0.667 Inf   -2.0279
##  Patersons Terrace Nov   overcast      0.2709 0.638 Inf   -1.7828
##  Snowy River       Dec   no cloud      0.5740 0.576 Inf   -1.2807
##  Snowy River       Jan   patchy cloud  0.6044 0.646 Inf   -1.4764
##  Snowy River       Jan   high cloud    0.6972 0.652 Inf   -1.4033
##  Patersons Terrace Jan   overcast      0.8483 0.636 Inf   -1.2010
##  Patersons Terrace Dec   patchy cloud  0.8770 0.681 Inf   -1.3146
##  Snowy River       Mar   no cloud      0.9683 0.557 Inf   -0.8249
##  Patersons Terrace Dec   high cloud    0.9698 0.648 Inf   -1.1180
##  Snowy River       Nov   no cloud      1.1581 0.601 Inf   -0.7782
##  Patersons Terrace Mar   patchy cloud  1.2712 0.677 Inf   -0.9090
##  Snowy River       Feb   patchy cloud  1.3112 0.671 Inf   -0.8480
##  Patersons Terrace Mar   high cloud    1.3640 0.674 Inf   -0.8065
##  Snowy River       Feb   high cloud    1.4040 0.646 Inf   -0.6770
##  Patersons Terrace Nov   patchy cloud  1.4610 0.695 Inf   -0.7767
##  Patersons Terrace Nov   high cloud    1.5538 0.715 Inf   -0.7475
##  Patersons Terrace Feb   overcast      1.5551 0.667 Inf   -0.5937
##  Snowy River       Jan   no cloud      1.7354 0.577 Inf   -0.1221
##  Patersons Terrace Dec   no cloud      2.0080 0.609 Inf    0.0462
##  Patersons Terrace Jan   patchy cloud  2.0384 0.680 Inf   -0.1518
##  Patersons Terrace Jan   high cloud    2.1312 0.692 Inf   -0.0965
##  Patersons Terrace Mar   no cloud      2.4022 0.590 Inf    0.5032
##  Snowy River       Feb   no cloud      2.4423 0.630 Inf    0.4134
##  Patersons Terrace Nov   no cloud      2.5920 0.637 Inf    0.5417
##  Patersons Terrace Feb   patchy cloud  2.7452 0.707 Inf    0.4679
##  Patersons Terrace Feb   high cloud    2.8380 0.690 Inf    0.6157
##  Patersons Terrace Jan   no cloud      3.1694 0.623 Inf    1.1633
##  Patersons Terrace Feb   no cloud      3.8762 0.677 Inf    1.6973
##  asymp.UCL .group
##      0.260  a
```

```
##      0.707  ab
##      0.933  abc
##      1.478  abcdefghijk
##      1.565  ab de g i
##      1.529  abcd fgh
##      1.637  abcdef   j l
##      1.951  abcdefghijklmnop
##      2.014  abcdefghijklmn  qrstuv
##      2.186  abcdefghijklm o q     wxyza
##      2.081  abcdefghijklm   q
##      2.325  abcdefghijklm   q  tu wx   zbzc
##      2.270   bcdefghijklmnop  s u w y  zb  zdzezfzg
##      2.325  abcdefghijklm   q  tu
##      2.429   bcdefghijklmnop  s u w y  zb  zdzezfzg
##      2.685   bcdefghijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzk
##      2.798   bcdefghijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzl
##      2.898   bcdefghijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzk
##      3.069  abcdefghijklmnopqr    wxyza    zdze    zhzi
##      2.761     defghijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzk  zm
##      3.058  abcdefghijklmn  qrstuvwx   zbzczd  zf  zh  zj
##      3.094     defghijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzk  zm
##      3.451  abcdefghijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzk  zm
##      3.470    c  f h jklmnopqrstuvwxyzazbzczdzezfzgzhzizjzk  zm
##      3.535  abcdefghijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmzn
##      3.485      e   ijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzk  zm
##      3.699   bcdefghijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmzn
##      3.855  abcdefghijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmzn
##      3.704       ghi k mnopqrstuvwxyzazbzczdzezfzgzhzizjzk  zm
##      3.593            lmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmzn
##      3.970           k mnopqrstuvwxyzazbzczdzezfzgzhzizjzk  zm
##      4.229        h  k mnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmzn
##      4.359         i k mnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmzn
##      4.301                     wxyzazbzczdzezfzgzhzizjzkzlzmzn
##      4.471               qr t v x za  zc        zhzizjzkzlzmzn
##      4.642              n p rs  v         zdzezfzgzhzizjzkzlzmzn
##      5.022                   stuv     zbzc    zfzg    zjzkzlzmzn
##      5.060               op        yza      ze  zg  zi  zkzlzmzn
##      5.175                                                 zmzn
##      6.055                                              zl  zn
##
## Results are given on the logit (not the response) scale.
## Confidence level used: 0.95
## Conf-level adjustment: sidak method for 40 estimates
## Results are given on the log odds ratio (not the response) scale.
## P value adjustment: tukey method for comparing a family of 40 estimates
## significance level used: alpha = 0.05

# ------------------------------
# Prepare data at 20m resolution
# ------------------------------
```

```
library(tidyverse)

## -- Attaching packages ---------------------------------------------------
------------------------------------------------------------ tidyverse 1.2.1
--

## v tibble  2.1.3     v purrr   0.2.5
## v tidyr   0.8.2     v stringr 1.3.1
## v readr   1.3.0     v forcats 0.3.0

## -- Conflicts ------------------------------------------------------------
------------------------------------------------- tidyverse_conflicts()
--
## x plyr::arrange()       masks dplyr::arrange()
## x data.table::between() masks dplyr::between()
## x purrr::compact()      masks plyr::compact()
## x plyr::count()         masks dplyr::count()
## x tidyr::expand()       masks Matrix::expand()
## x plyr::failwith()      masks dplyr::failwith()
## x dplyr::filter()       masks stats::filter()
## x data.table::first()   masks dplyr::first()
## x plyr::id()            masks dplyr::id()
## x dplyr::lag()          masks stats::lag()
## x data.table::last()    masks dplyr::last()
## x plyr::mutate()        masks dplyr::mutate()
## x plyr::rename()        masks dplyr::rename()
## x dplyr::select()       masks MASS::select()
## x plyr::summarise()     masks dplyr::summarise()
## x plyr::summarize()     masks dplyr::summarize()
## x purrr::transpose()    masks data.table::transpose()

#read in the data
dat<-read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Combined data filtered.csv',na.strings = "")

#change the names of the factors
library(plyr)
dat$Location <- revalue(dat$Location, c("Pattersons"="Patersons Terrace",
"Snowy"="Snowy River"))

# drop columns that aren't needed
dat <- dat %>%

select_("Sex","Visit_All_time","Location","Transect","Season","Month","Ground
","Baro", "Wind", "Sky", "Standard_time","X20mtransect")

#make X20mtransect a factor
dat$X20mtransect <- as.factor(as.integer(dat$X20mtransect))
```

```r
#create a new dataset where there is data for each 20m searched.

# create a new object with the different categories in it
skel <- data.frame(X20mtransect = seq(20, 100, 20))
skel$X20mtransect <- as.factor(skel$X20mtransect)

#form the new dataset by joining them together
dat1 <- dat %>%
  group_by_at(vars(Visit_All_time:Standard_time)) %>%
  nest() %>%
  mutate(data = map(data, ~full_join(.x, skel, by = "X20mtransect"))) %>%
  unnest() %>%
  replace_na(list(Present = 0))

# make the NAs in the sex column a '0'
dat1$Sex[is.na (dat1$Sex)] <- 0

#create new column with 0 or 1 or represent when a grasshopper is present
dat1$Present <- (dat1$Sex != "0")
dat1$Present <- as.integer(as.logical(dat1$Present))

#add time squared variable
dat1$time_sq <- dat1$Standard_time*dat1$Standard_time

#make column with standardised ground temperature
dat1$Ground <- as.numeric(as.character(dat1$Ground))
dat1$Ground_standard <- (dat1$Ground - mean(dat1$Ground, na.rm = T)) /
sd(dat1$Ground, na.rm = T)
dat1$temp_sq <- dat1$Ground_standard*dat1$Ground_standard

#rename the transects so that snowy and patersons have different IDs
dat1$TransectID <- paste(dat1$Location, dat1$Transect,sep="-")

#make the new column a factor rather than a character
dat1$TransectID <- as.factor(dat1$TransectID)

#rescale season so thats its on a better scale
dat1$SeasonID <- dat1$Season - 2015

#subset for unique rows of info
library(data.table)
dt <- data.table(dat1)
dt1<-dt[,.SD[which.max(Present)],by=list(TransectID, Visit_All_time,
X20mtransect)]

#remove April data
dt1<-dt1[(dt1$Month != "Apr"),]

#drop rows where temp and sky contain NA
```

```
dt1 <- dt1[dt1$Ground != 'NA',]
dt1 <- dt1[dt1$Sky != 'NA',]
dt1 <- droplevels(dt1)

#check for missing values
library(Amelia)
missmap(dt1, main = "Missing values vs observed")
```

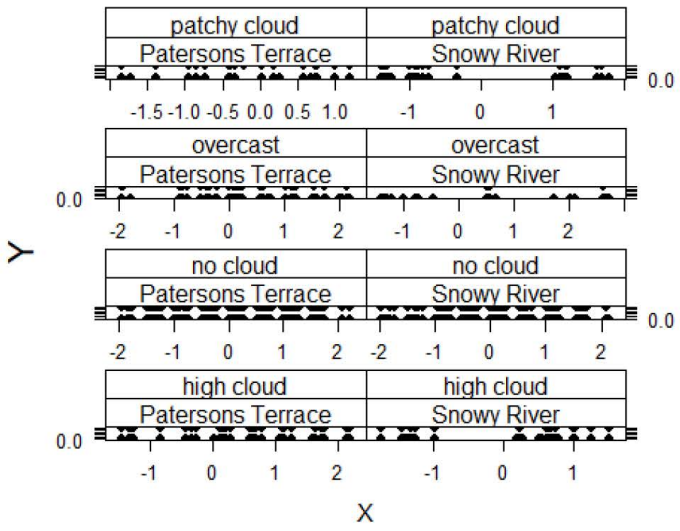



```
#drop excess levels from the dataframe
droplevels(dt1)
```

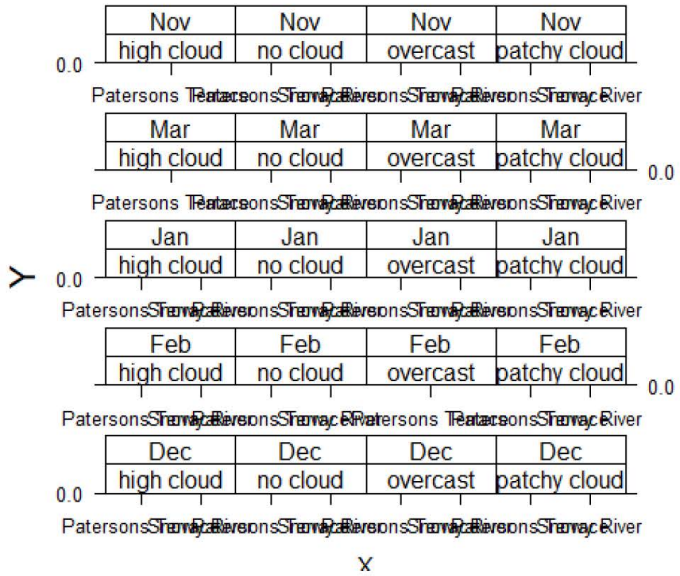
```
##                TransectID Visit_All_time X20mtransect          Location
##    1: Patersons Terrace-A              6           20 Patersons Terrace
##    2: Patersons Terrace-A              6          100 Patersons Terrace
##    3: Patersons Terrace-A              6           40 Patersons Terrace
##    4: Patersons Terrace-A              6           60 Patersons Terrace
##    5: Patersons Terrace-A              6           80 Patersons Terrace
##   ---
## 2361:       Snowy River-D             56          100       Snowy River
## 2362:       Snowy River-D             56           20       Snowy River
## 2363:       Snowy River-D             56           40       Snowy River
## 2364:       Snowy River-D             56           60       Snowy River
## 2365:       Snowy River-D             56           80       Snowy River
##       Transect Season Month Ground   Baro   Wind      Sky Standard_time
##    1:        A   2015   Dec   35.1  988.5  still no cloud         0.050
##    2:        A   2015   Dec   35.1  988.5  still no cloud         0.050
##    3:        A   2015   Dec   35.1  988.5  still no cloud         0.050
```

```
##    4:        A   2015   Dec   26.0  982.5 breeze no cloud        -0.775
##    5:        A   2015   Dec   35.1  988.5  still no cloud         0.050
##   ---
## 2361:        D   2017   Mar   26.8 1039.9  still no cloud        -0.830
## 2362:        D   2017   Mar   26.8 1039.9  still no cloud        -0.830
## 2363:        D   2017   Mar   26.8 1039.9  still no cloud        -0.830
## 2364:        D   2017   Mar   26.8 1039.9  still no cloud        -0.830
## 2365:        D   2017   Mar   26.8 1039.9  still no cloud        -0.830
##          Sex Present  time_sq Ground_standard     temp_sq SeasonID
##    1: female       1 0.002500      1.46064193 2.133474852        0
##    2:   male       1 0.002500      1.46064193 2.133474852        0
##    3:      0       0 0.002500      1.46064193 2.133474852        0
##    4:  nymph       1 0.600625      0.05211454 0.002715925        0
##    5:      0       0 0.002500      1.46064193 2.133474852        0
##   ---
## 2361:      0       0 0.688900      0.17594112 0.030955278        2
## 2362:      0       0 0.688900      0.17594112 0.030955278        2
## 2363:      0       0 0.688900      0.17594112 0.030955278        2
## 2364:      0       0 0.688900      0.17594112 0.030955278        2
## 2365:      0       0 0.688900      0.17594112 0.030955278        2
```

```r
dat20 <- dt1

# ------------------------------
# Logistic regression on 20m data
# ------------------------------

#variables of interest: time, time_sq, location, transect ID (random!),
sky/cloud cover, temperature, temperature_sq, season
# note that there is no temperature for the November data

#Explore the dataset

#create graph
library(lattice)
xyplot(Present ~ Standard_time | Location*Sky,
       data = dat20, pch = 16, col = 1,
       strip = function(bg = "white", ...)
         strip.default(bg = 'white', ...),
       scales = list(alternating = TRUE,
                     x = list(relation = "free"),
                     y = list(relation = "same")),
       xlab = list(label = "X", ex = 1.5),
       ylab = list(label = "Y", cex = 1.5))
```




```
xyplot(Present ~ Location | Sky*Month,
       data = dat20, pch = 16, col = 1,
       strip = function(bg = "white", ...)
         strip.default(bg = 'white', ...),
       scales = list(alternating = TRUE,
                     x = list(relation = "free"),
                     y = list(relation = "same")),
       xlab = list(label = "X", ex = 1.5),
       ylab = list(label = "Y", cex = 1.5))
```




```
#Look at the distribution of 0s and 1s
table(dat20$Present, dat20$Location, dat20$Month)
```

```
## , ,  = Dec
##
##
##     Patersons Terrace Snowy River
##   0               176         217
##   1                64          23
##
## , ,  = Feb
##
##
##     Patersons Terrace Snowy River
##   0               156         145
##   1               109          65
##
## , ,  = Jan
##
##
##     Patersons Terrace Snowy River
##   0               171         159
##   1               144          61
##
## , ,  = Mar
##
##
```

```
##     Patersons Terrace Snowy River
##   0               182         192
##   1               118          63
##
## , ,  = Nov
##
##
##     Patersons Terrace Snowy River
##   0                92         162
##   1                43          23
```

```
#run logistic regression
library(lme4)
library(lmerTest)

#full model
mod1 <- glmer(Present ~ Location + Month + Sky + Ground_standard +
(1|SeasonID) + (1|TransectID) ,  family = "binomial", data = dat20)

#basic informative model
mod2 <- glmer(Present ~ Location + Month + Sky + (1|SeasonID) +
(1|TransectID),  family = "binomial", data = dat20)

mod3 <- glmer(Present ~ Location + Month + (1|SeasonID) + (1|TransectID),
family = "binomial", data = dat20)

mod4 <- glmer(Present ~ Location + Sky + (1|SeasonID) + (1|TransectID),
family = "binomial", data = dat20)

anova(mod1, mod2, mod3, mod4)
```

```
## Data: dat20
## Models:
## mod4: Present ~ Location + Sky + (1 | SeasonID) + (1 | TransectID)
## mod3: Present ~ Location + Month + (1 | SeasonID) + (1 | TransectID)
## mod2: Present ~ Location + Month + Sky + (1 | SeasonID) + (1 | TransectID)
## mod1: Present ~ Location + Month + Sky + Ground_standard + (1 | SeasonID)
+
## mod1:     (1 | TransectID)
##      Df    AIC    BIC  logLik deviance   Chisq Chi Df Pr(>Chisq)
## mod4  7 2683.0 2723.4 -1334.5   2669.0
## mod3  8 2709.3 2755.5 -1346.7   2693.3  0.0000      1     1.0000
## mod2 11 2649.0 2712.4 -1313.5   2627.0 66.3330      3  2.601e-14 ***
## mod1 12 2650.0 2719.2 -1313.0   2626.0  1.0043      1     0.3163
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
AIC(mod1, mod2, mod3, mod4)
```

```
##      df      AIC
## mod1 12 2649.982
## mod2 11 2648.987
## mod3  8 2709.320
## mod4  7 2683.040

#model 2 comes out on top

#check for overdispersion
E1 <- residuals(mod2)
p1 <- length(fixef(mod2)) + 1
(overdisp1 <- sum(E1^2) /(nrow(dat20) - p1))

## [1] 1.103504

# value has to be less than 1 (there is evidence of overdispersion in this
model)
# However, overdispersion not a big issue for binomial mixed effects model.

summary(mod2)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: binomial  ( logit )
## Formula:
## Present ~ Location + Month + Sky + (1 | SeasonID) + (1 | TransectID)
##    Data: dat20
##
##      AIC      BIC   logLik deviance df.resid
##   2649.0   2712.4  -1313.5   2627.0     2354
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.2610 -0.6522 -0.4360  0.9201  3.4510
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  TransectID (Intercept) 0.0805   0.2837
##  SeasonID   (Intercept) 0.1472   0.3837
## Number of obs: 2365, groups:  TransectID, 8; SeasonID, 3
##
## Fixed effects:
##                     Estimate Std. Error z value Pr(>|z|)
## (Intercept)         -1.22392    0.33033  -3.705 0.000211 ***
## LocationSnowy River -0.76456    0.23832  -3.208 0.001336 **
## MonthFeb             0.86686    0.15985   5.423 5.87e-08 ***
## MonthJan             0.82243    0.15567   5.283 1.27e-07 ***
## MonthMar             0.55222    0.15708   3.516 0.000439 ***
## MonthNov             0.28672    0.19162   1.496 0.134572
## Skyno cloud          0.44184    0.15472   2.856 0.004294 **
## Skyovercast         -0.79767    0.21195  -3.764 0.000168 ***
```

```
## Skypatchy cloud      0.05711    0.20938   0.273 0.785024
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) LctnSR MnthFb MnthJn MnthMr MnthNv Skyncl Skyvrc
## LctnSnwyRvr -0.404
## MonthFeb    -0.292 -0.002
## MonthJan    -0.289  0.008  0.620
## MonthMar    -0.274 -0.003  0.606  0.631
## MonthNov    -0.205 -0.001  0.496  0.513  0.510
## Skyno cloud -0.377  0.033 -0.002 -0.044 -0.074 -0.079
## Skyovercast -0.312  0.055  0.060  0.056  0.040 -0.038  0.614
## Skyptchycld -0.267 -0.004 -0.036 -0.047 -0.014 -0.062  0.623  0.451
```

```
# ------------
# Plot model 2
# ------------

#note: make sure the order of the months is alphabetical as it is specified
in the model.
NewData <- expand.grid(Location = levels(dat20$Location),
                       Month = levels(dat20$Month),
                       Sky = levels(dat20$Sky),
                       SeasonID = c(0:20),
                       TransectID = levels(dat20$TransectID))

X <- model.matrix(~ Location + Month + Sky,
                  data = NewData)

NewData$eta         <- X %*% fixef(mod2)
NewData$SuccessPred <- exp(NewData$eta) / (1 + exp(NewData$eta))
head(NewData,15)
```

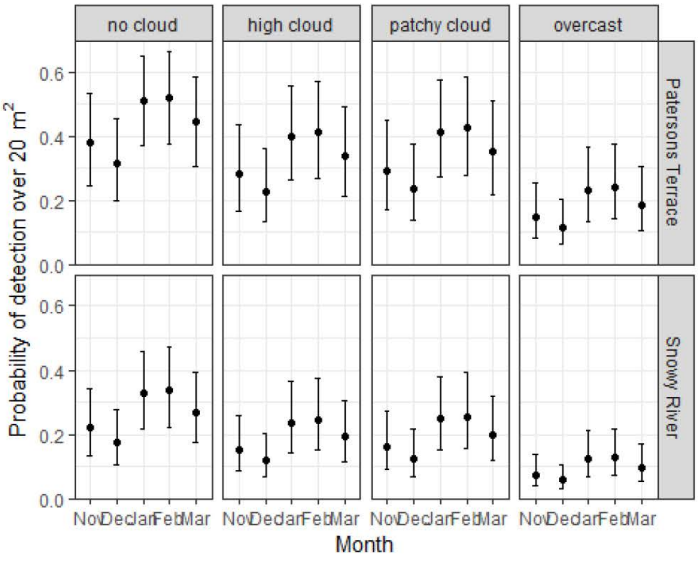
```
##             Location Month        Sky SeasonID          TransectID
## 1  Patersons Terrace   Dec high cloud        0 Patersons Terrace-A
## 2         Snowy River   Dec high cloud        0 Patersons Terrace-A
## 3  Patersons Terrace   Feb high cloud        0 Patersons Terrace-A
## 4         Snowy River   Feb high cloud        0 Patersons Terrace-A
## 5  Patersons Terrace   Jan high cloud        0 Patersons Terrace-A
## 6         Snowy River   Jan high cloud        0 Patersons Terrace-A
## 7  Patersons Terrace   Mar high cloud        0 Patersons Terrace-A
## 8         Snowy River   Mar high cloud        0 Patersons Terrace-A
## 9  Patersons Terrace   Nov high cloud        0 Patersons Terrace-A
## 10        Snowy River   Nov high cloud        0 Patersons Terrace-A
## 11 Patersons Terrace   Dec   no cloud        0 Patersons Terrace-A
## 12        Snowy River   Dec   no cloud        0 Patersons Terrace-A
## 13 Patersons Terrace   Feb   no cloud        0 Patersons Terrace-A
## 14        Snowy River   Feb   no cloud        0 Patersons Terrace-A
## 15 Patersons Terrace   Jan   no cloud        0 Patersons Terrace-A
```

```
##            eta SuccessPred
## 1  -1.22391685   0.2272479
## 2  -1.98848005   0.1204178
## 3  -0.35705900   0.4116717
## 4  -1.12162220   0.2457105
## 5  -0.40148466   0.4009557
## 6  -1.16604786   0.2375701
## 7  -0.67169540   0.3381173
## 8  -1.43625860   0.1921254
## 9  -0.93719631   0.2814670
## 10 -1.70175951   0.1542356
## 11 -0.78207560   0.3138727
## 12 -1.54663880   0.1755723
## 13  0.08478225   0.5211829
## 14 -0.67978095   0.3363102
## 15  0.04035659   0.5100878
```

```
#Get the standard errors
NewData$VarPred <- diag(X %*% vcov(mod2) %*% t(X))
NewData$sePred  <- sqrt(NewData$VarPred)

NewData$seLow  <- exp(NewData$eta - 1.96 * NewData$sePred) / (1 +
exp(NewData$eta - 1.96 * NewData$sePred))
NewData$seHigh <- exp(NewData$eta + 1.96 * NewData$sePred) / (1 +
exp(NewData$eta + 1.96 * NewData$sePred))
head(NewData, 10)
```

```
##             Location Month        Sky SeasonID          TransectID
## 1  Patersons Terrace   Dec high cloud        0 Patersons Terrace-A
## 2        Snowy River   Dec high cloud        0 Patersons Terrace-A
## 3  Patersons Terrace   Feb high cloud        0 Patersons Terrace-A
## 4        Snowy River   Feb high cloud        0 Patersons Terrace-A
## 5  Patersons Terrace   Jan high cloud        0 Patersons Terrace-A
## 6        Snowy River   Jan high cloud        0 Patersons Terrace-A
## 7  Patersons Terrace   Mar high cloud        0 Patersons Terrace-A
## 8        Snowy River   Mar high cloud        0 Patersons Terrace-A
## 9  Patersons Terrace   Nov high cloud        0 Patersons Terrace-A
## 10       Snowy River   Nov high cloud        0 Patersons Terrace-A
##           eta SuccessPred    VarPred    sePred      seLow    seHigh
## 1  -1.2239168   0.2272479 0.10911640 0.3303277 0.13338453 0.3597442
## 2  -1.9884800   0.1204178 0.10232328 0.3198801 0.06815099 0.2039942
## 3  -0.3570590   0.4116717 0.10382793 0.3222234 0.27118535 0.5681970
## 4  -1.1216222   0.2457105 0.09688479 0.3112632 0.15037050 0.3748311
## 5  -0.4014847   0.4009557 0.10361894 0.3218990 0.26261780 0.5571090
## 6  -1.1660479   0.2375701 0.09738533 0.3120662 0.14458766 0.3648446
## 7  -0.6716954   0.3381173 0.10538245 0.3246266 0.21282781 0.4911441
## 8  -1.4362586   0.1921254 0.09833830 0.3135894 0.11396210 0.3054192
## 9  -0.9371963   0.2814670 0.11992717 0.3463050 0.16576360 0.4357476
## 10 -1.7017595   0.1542356 0.11304621 0.3362234 0.08621450 0.2606175
```

```
#reorder the levels so that they plto correctly
NewData$Month2 <- factor(NewData$Month, levels = c("Nov", "Dec", "Jan",
"Feb", "Mar"))
NewData$Sky2 <- factor(NewData$Sky, levels = c("no cloud", "high cloud",
"patchy cloud", "overcast"))

#rename levels
levels(NewData$Location)[levels(NewData$Location == "Pattersons")] <-
"Patersons Terrace"
levels(NewData$Location)[levels(NewData$Location == "Snowy")] <- "Snowy
River"

dat20 <- NewData

library(ggplot2)

ggplot(data = NewData, aes(Month2, SuccessPred)) +
  geom_point() + #plot the points
  ylab("Probability detection (20m transect)") +
  theme_bw() +
  geom_errorbar(aes(ymin=seLow, ymax=seHigh), width = 0.2) +
  facet_grid(Location ~ Sky2) +
  labs(x = "Month", y = (expression("Probability of detection over 20
"~m^2)))
```

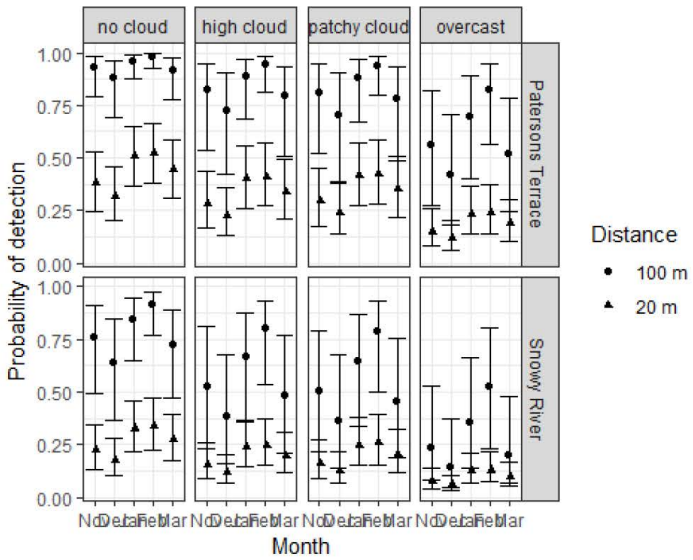


```
# -------------
# post-hoc test
# --------------

marginal20 <- lsmeans(mod2, ~ Location + Month + Sky, adjust = "tukey")

(post_hoc_20 <- cld(marginal20,
                    alpha = 0.05,
                    Letters = letters,
                    adjust = "tukey"))

##  Location          Month Sky           lsmean    SE  df asymp.LCL
##  Snowy River       Dec   overcast     -2.7861 0.330 Inf    -3.850
##  Snowy River       Nov   overcast     -2.4994 0.342 Inf    -3.600
##  Snowy River       Mar   overcast     -2.2339 0.328 Inf    -3.291
##  Patersons Terrace Dec   overcast     -2.0216 0.332 Inf    -3.091
##  Snowy River       Dec   high cloud   -1.9885 0.320 Inf    -3.019
##  Snowy River       Jan   overcast     -1.9637 0.329 Inf    -3.022
##  Snowy River       Dec   patchy cloud -1.9314 0.330 Inf    -2.994
##  Snowy River       Feb   overcast     -1.9193 0.328 Inf    -2.976
##  Patersons Terrace Nov   overcast     -1.7349 0.344 Inf    -2.841
##  Snowy River       Nov   high cloud   -1.7018 0.336 Inf    -2.784
##  Snowy River       Nov   patchy cloud -1.6446 0.339 Inf    -2.735
##  Snowy River       Dec   no cloud     -1.5466 0.300 Inf    -2.514
##  Patersons Terrace Mar   overcast     -1.4694 0.331 Inf    -2.534
##  Snowy River       Mar   high cloud   -1.4363 0.314 Inf    -2.446
##  Snowy River       Mar   patchy cloud -1.3791 0.322 Inf    -2.418
##  Snowy River       Nov   no cloud     -1.2599 0.310 Inf    -2.259
##  Patersons Terrace Dec   high cloud   -1.2239 0.330 Inf    -2.288
##  Patersons Terrace Jan   overcast     -1.1992 0.330 Inf    -2.260
##  Patersons Terrace Dec   patchy cloud -1.1668 0.341 Inf    -2.264
##  Snowy River       Jan   high cloud   -1.1660 0.312 Inf    -2.171
##  Patersons Terrace Feb   overcast     -1.1547 0.330 Inf    -2.218
##  Snowy River       Feb   high cloud   -1.1216 0.311 Inf    -2.124
##  Snowy River       Jan   patchy cloud -1.1089 0.318 Inf    -2.132
##  Snowy River       Feb   patchy cloud -1.0645 0.318 Inf    -2.088
##  Snowy River       Mar   no cloud     -0.9944 0.287 Inf    -1.920
##  Patersons Terrace Nov   high cloud   -0.9372 0.346 Inf    -2.052
##  Patersons Terrace Nov   patchy cloud -0.8801 0.349 Inf    -2.004
##  Patersons Terrace Dec   no cloud     -0.7821 0.307 Inf    -1.772
##  Snowy River       Jan   no cloud     -0.7242 0.288 Inf    -1.653
##  Snowy River       Feb   no cloud     -0.6798 0.291 Inf    -1.617
##  Patersons Terrace Mar   high cloud   -0.6717 0.325 Inf    -1.717
##  Patersons Terrace Mar   patchy cloud -0.6146 0.334 Inf    -1.690
##  Patersons Terrace Nov   no cloud     -0.4954 0.317 Inf    -1.517
##  Patersons Terrace Jan   high cloud   -0.4015 0.322 Inf    -1.438
##  Patersons Terrace Feb   high cloud   -0.3571 0.322 Inf    -1.395
##  Patersons Terrace Jan   patchy cloud -0.3444 0.328 Inf    -1.400
##  Patersons Terrace Feb   patchy cloud -0.2999 0.329 Inf    -1.360
##  Patersons Terrace Mar   no cloud     -0.2299 0.295 Inf    -1.181
```

```
##  Patersons Terrace Jan   no cloud      0.0404 0.295 Inf    -0.909
##  Patersons Terrace Feb   no cloud      0.0848 0.299 Inf    -0.877
##  asymp.UCL
##    -1.7224
##    -1.3990
##    -1.1766
##    -0.9518
##    -0.9584
##    -0.9059
##    -0.8688
##    -0.8624
##    -0.6282
##    -0.6191
##    -0.5546
##    -0.5796
##    -0.4048
##    -0.4265
##    -0.3407
##    -0.2609
##    -0.1602
##    -0.1380
##    -0.0697
##    -0.1612
##    -0.0910
##    -0.1193
##    -0.0862
##    -0.0411
##    -0.0689
##     0.1779
##     0.2441
##     0.2081
##     0.2042
##     0.2571
##     0.3736
##     0.4604
##     0.5265
##     0.6351
##     0.6805
##     0.7115
##     0.7600
##     0.7211
##     0.9897
##     1.0463
##  .group
##   a
##   ab
##   abcd
##   abc efgh
##   abcd f  ijk
##    bcdefg ijklmno
```

```
##   abcde   i  lm
##    bcdefg ijklmno
##   abcdefghij l n pqr
##   abcdefghijklmnop  stu
##    bcdefghijklmnopq s  vwxyza
##    bcdefghijklmnop  stu w y  zbzczdzezfzgzhzizj
##    bcdefghijklmnopqrs  v     zb                zk
##     cdefghijklmnopqrstuvwx   zbzczd  zfzg  zi  zkzlzmznzozp
##     cdefghijklmnopqrst vwxyzazbzczdzezf  zh    zkzlzmzn    zqzr
##     cdefghijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzszt
##    bcdefghijklmnopqrstuv     zb      zf    zi  zk  zm  zo
##      d    ijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzs
##    bcdefghijklmnopqrs  vwxyzazbzc  ze          zkzl        zq
##       e gh   lmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzszt
##           ijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzs
##       e gh   lmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzsztzu
##        fgh jk  nopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzszt  zv
##        fgh jk  nopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzsztzu
##          h       pqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzsztzu
##     cdefghijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzszt
##      d    ijklmnopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzsztzu
##                nopqrstuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzszt
##                   qr   v x za                zkzlzmznzozpzqzrzsztzuzvzw
##                    r                         zkzlzmznzozpzqzrzsztzuzvzw
##               m o   stuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzsztzuzvzw
##             k   o   stuvwxyzazbzczdzezfzgzhzizjzkzlzmznzozpzqzrzsztzuzvzw
##                           zbzczdzezfzgzhzizjzkzlzmznzozpzqzrzsztzuzvzw
##                        wxyza  zczdze  zgzh  zj  zl  zn  zpzqzrzsztzuzvzw
##                          yza      ze    zh  zj           zqzrzsztzuzvzw
##                     tu          zd  zfzgzhzizj    zmznzozp  zrzsztzuzvzw
##                      u               zg  zizj        zozp    zsztzuzvzw
##                                                            ztzuzvzw
##                                                              zu  zw
##                                                                zvzw
##
## Results are given on the logit (not the response) scale.
## Confidence level used: 0.95
## Conf-level adjustment: sidak method for 40 estimates
## Results are given on the log odds ratio (not the response) scale.
## P value adjustment: tukey method for comparing a family of 40 estimates
## significance level used: alpha = 0.05

# --------------------
# make combined graph
# --------------------

#join dat20 and dat100 together

dat20$Distance <- c("20 m")
dat100$Distance <- c("100 m")
```

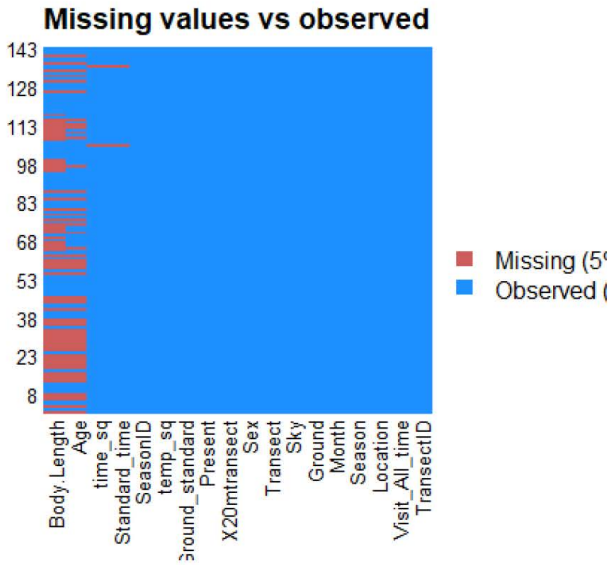
```
dat.all <- rbind(dat20,dat100)

#plot the graph

dodge <- position_dodge(width=0.5)

ggplot(data = dat.all, aes(Month2, SuccessPred)) +
  geom_point(aes(shape=Distance),position=position_dodge(width = 0.2)) +
#plot the points
  ylab("Probability detection") +
  theme_bw() +
  geom_errorbar(aes(ymin=seLow, ymax=seHigh), position = "dodge") +
  facet_grid(Location ~ Sky2) +
  labs(x = "Month", y = (expression("Probability of detection")))
```




```
# ----------------------------------------------------
# Probability of detecting adult females grasshopper
# ----------------------------------------------------

# -------------------------------------
# Prepare the data at a 100m resolution
# -------------------------------------

library(tidyverse)
```

```
#read in the data
dat<-read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Combined data filtered.csv',na.strings = "")

#subset data to get only Nov and Dec
dat<-dat[dat$Month =='Nov' | dat$Month == 'Dec',]

#fix age variable to only have 3 levels
dat$Age[dat$Age == "J" | dat$Age == "nymph"] <- "j"
dat$Age[dat$Age == "no data"] <- NA
dat[dat == "no data"] <- NA
dat <- droplevels(dat)


#drop columns that aren't of interest
library(dplyr)
dat1 <- dat %>%
  mutate_all(as.factor) %>%
  select(Location, Season, Month, Visit_All_time, Ground, Sky, Standard_time,
Transect, Sex, Body.Length, Age, X20mtransect)

#create new column with 0 or 1 or represent when adult females present
dat1$Present <- (dat1$Sex == "female" & dat1$Age == "a")
dat1$Present <- as.integer(as.logical(dat1$Present))

#add time squared variable
dat1$Standard_time <- as.numeric(paste(dat1$Standard_time))
dat1$time_sq <- dat1$Standard_time*dat1$Standard_time

#make column with standardised ground temperature
dat1$Ground <- as.numeric(as.character(dat1$Ground))
dat1$Ground_standard <- (dat1$Ground - mean(dat1$Ground, na.rm = T)) /
sd(dat1$Ground, na.rm = T)
dat1$temp_sq <- dat1$Ground_standard*dat1$Ground_standard

#rename the transects so that snowy and patersons have different IDs
dat1$TransectID <- paste(dat1$Location, dat1$Transect,sep="-")

#make the new column a factor rather than a character
dat1$TransectID <- as.factor(dat1$TransectID)

#rescale season so thats its on a better scale
dat1$Season <- as.numeric(paste(dat$Season))
dat1$SeasonID <- dat1$Season - 2015

#subset for unique rows of info
library(data.table)
dt <- data.table(dat1)
dt1<-dt[,.SD[which.max(Present)],by=list(TransectID, Visit_All_time)]
```

```
#drop rows where temp and sky contain NA
dt1 <- dt1[dt1$Ground != 'NA',]
dt1 <- dt1[dt1$Sky != 'NA',]
dt1 <- droplevels(dt1)

#check for missing values
library(Amelia)
missmap(dt1, main = "Missing values vs observed")
```




```
# ----------------------
# Detectability at 100 m
# ----------------------

#Look at the distribution of 0s and 1s
table(dt1$Present, dt1$Location)

##
##     Pattersons Snowy
##   0         68    58
##   1          7    10

dt1$TransectID <- as.factor(as.character(dt1$TransectID))

#run logistic regression
library(lme4)
library(lmerTest)
```

```r
# modelling

mod2 <- glmer(Present ~ Location + Sky + (1|SeasonID) + (1|TransectID),
family = "binomial", data = dt1)

## singular fit

mod3 <- glmer(Present ~ Location + (1|SeasonID) + (1|TransectID),  family =
"binomial", data = dt1)

## singular fit

mod4 <- glmer(Present ~ Sky  + (1|SeasonID) + (1|TransectID),  family =
"binomial", data = dt1)

## singular fit

anova(mod2, mod3)

## Data: dt1
## Models:
## mod3: Present ~ Location + (1 | SeasonID) + (1 | TransectID)
## mod2: Present ~ Location + Sky + (1 | SeasonID) + (1 | TransectID)
##      Df    AIC    BIC  logLik deviance Chisq Chi Df Pr(>Chisq)
## mod3  4 111.32 123.17 -51.659   103.32
## mod2  7 117.03 137.77 -51.517   103.03 0.284      3      0.963

anova(mod2,mod4)

## Data: dt1
## Models:
## mod4: Present ~ Sky + (1 | SeasonID) + (1 | TransectID)
## mod2: Present ~ Location + Sky + (1 | SeasonID) + (1 | TransectID)
##      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## mod4  6 115.75 133.53 -51.877   103.75
## mod2  7 117.03 137.77 -51.517   103.03 0.7204      1      0.396

AIC(mod2, mod3, mod4)

##      df      AIC
## mod2  7 117.0334
## mod3  4 111.3174
## mod4  6 115.7538

# models do not significantly differ, model 3 has lowest AIC

# check model fit

E1 <- residuals(mod3)
p1 <- length(fixef(mod3)) + 1
(overdisp1 <- sum(E1^2) /(nrow(dt1) - p1))
```

```
## [1] 0.7379813

summary(mod3)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: binomial  ( logit )
## Formula: Present ~ Location + (1 | SeasonID) + (1 | TransectID)
##    Data: dt1
##
##      AIC      BIC   logLik deviance df.resid
##    111.3    123.2    -51.7    103.3      139
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.4152 -0.4152 -0.3208 -0.3208  3.1168
##
## Random effects:
##  Groups     Name        Variance  Std.Dev.
##  TransectID (Intercept) 4.419e-14 2.102e-07
##  SeasonID   (Intercept) 0.000e+00 0.000e+00
## Number of obs: 143, groups:  TransectID, 8; SeasonID, 3
##
## Fixed effects:
##               Estimate Std. Error z value Pr(>|z|)
## (Intercept)    -2.2736     0.3969  -5.728 1.02e-08 ***
## LocationSnowy   0.5157     0.5242   0.984    0.325
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr)
## LocatinSnwy -0.757
## convergence code: 0
## singular fit

# ---------------
# Plot the model
# ---------------

#note: make sure the order of the months is alphabetical as it is specified
in the model.
NewData <- expand.grid(Location = levels(dt1$Location),
                       SeasonID = c(0:20),
                       Month = levels(dt1$Month),
                       TransectID = levels(dt1$TransectID))

head(NewData)

##     Location SeasonID Month   TransectID
## 1 Pattersons        0   Dec Pattersons-A
```

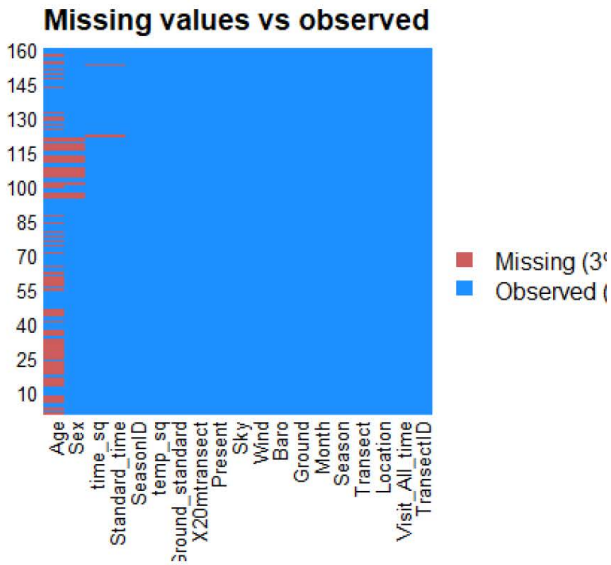
```
## 2      Snowy        0   Dec Pattersons-A
## 3 Pattersons        1   Dec Pattersons-A
## 4      Snowy        1   Dec Pattersons-A
## 5 Pattersons        2   Dec Pattersons-A
## 6      Snowy        2   Dec Pattersons-A

X <- model.matrix(~ Location,
                  data = NewData)

NewData$eta         <- X %*% fixef(mod3)
NewData$SuccessPred <- exp(NewData$eta) / (1 + exp(NewData$eta))
head(NewData,15)

##      Location SeasonID Month   TransectID       eta SuccessPred
## 1  Pattersons        0   Dec Pattersons-A -2.273598  0.09333333
## 2       Snowy        0   Dec Pattersons-A -1.757858  0.14705882
## 3  Pattersons        1   Dec Pattersons-A -2.273598  0.09333333
## 4       Snowy        1   Dec Pattersons-A -1.757858  0.14705882
## 5  Pattersons        2   Dec Pattersons-A -2.273598  0.09333333
## 6       Snowy        2   Dec Pattersons-A -1.757858  0.14705882
## 7  Pattersons        3   Dec Pattersons-A -2.273598  0.09333333
## 8       Snowy        3   Dec Pattersons-A -1.757858  0.14705882
## 9  Pattersons        4   Dec Pattersons-A -2.273598  0.09333333
## 10      Snowy        4   Dec Pattersons-A -1.757858  0.14705882
## 11 Pattersons        5   Dec Pattersons-A -2.273598  0.09333333
## 12      Snowy        5   Dec Pattersons-A -1.757858  0.14705882
## 13 Pattersons        6   Dec Pattersons-A -2.273598  0.09333333
## 14      Snowy        6   Dec Pattersons-A -1.757858  0.14705882
## 15 Pattersons        7   Dec Pattersons-A -2.273598  0.09333333

#Get the standard errors
NewData$VarPred <- diag(X %*% vcov(mod3) %*% t(X))
NewData$sePred  <- sqrt(NewData$VarPred)

NewData$seLow  <- exp(NewData$eta - 1.96 * NewData$sePred) / (1 +
exp(NewData$eta - 1.96 * NewData$sePred))
NewData$seHigh <- exp(NewData$eta + 1.96 * NewData$sePred) / (1 +
exp(NewData$eta + 1.96 * NewData$sePred))
head(NewData, 10)

##      Location SeasonID Month   TransectID       eta SuccessPred   VarPred
## 1  Pattersons        0   Dec Pattersons-A -2.273598  0.09333333 0.1575624
## 2       Snowy        0   Dec Pattersons-A -1.757858  0.14705882 0.1172412
## 3  Pattersons        1   Dec Pattersons-A -2.273598  0.09333333 0.1575624
## 4       Snowy        1   Dec Pattersons-A -1.757858  0.14705882 0.1172412
## 5  Pattersons        2   Dec Pattersons-A -2.273598  0.09333333 0.1575624
## 6       Snowy        2   Dec Pattersons-A -1.757858  0.14705882 0.1172412
## 7  Pattersons        3   Dec Pattersons-A -2.273598  0.09333333 0.1575624
## 8       Snowy        3   Dec Pattersons-A -1.757858  0.14705882 0.1172412
## 9  Pattersons        4   Dec Pattersons-A -2.273598  0.09333333 0.1575624
## 10      Snowy        4   Dec Pattersons-A -1.757858  0.14705882 0.1172412
```

```
##       sePred      seLow    seHigh
## 1  0.3969413 0.04514834 0.1830838
## 2  0.3424050 0.08098997 0.2522319
## 3  0.3969413 0.04514834 0.1830838
## 4  0.3424050 0.08098997 0.2522319
## 5  0.3969413 0.04514834 0.1830838
## 6  0.3424050 0.08098997 0.2522319
## 7  0.3969413 0.04514834 0.1830838
## 8  0.3424050 0.08098997 0.2522319
## 9  0.3969413 0.04514834 0.1830838
## 10 0.3424050 0.08098997 0.2522319
```

```
#reorder the levels so that they plot correctly
NewData$Month2 <- factor(NewData$Month, levels = c("Nov", "Dec", "Jan",
"Feb", "Mar"))


dat100 <- NewData
dat100$Location <- revalue(dat100$Location, c("Pattersons"="Patersons
Terrace", "Snowy"="Snowy River"))

# ------------------------------
# Prepare data at 20m resolution
# ------------------------------

#read in the data
dat<-read.csv('C:/Users/Jennifer/Desktop/Data Chapters/Transect
monitoring/Combined data filtered.csv',na.strings = "")

#change the names of the factors
dat$Location <- revalue(dat$Location, c("Pattersons"="Patersons Terrace",
"Snowy"="Snowy River"))

# drop columns that aren't needed
dat <- dat %>%
  select(Sex,Age, Visit_All_time,Location,Transect,Season,Month,Ground,Baro,
Wind, Sky, Standard_time,X20mtransect)

#make X20mtransect a factor
dat$X20mtransect <- as.factor(as.integer(dat$X20mtransect))

#fix age variable to only have 3 levels
dat$Age[dat$Age == "J" | dat$Age == "nymph"] <- "j"
dat$Age[dat$Age == "no data"] <- NA
dat[dat == "no data"] <- NA
dat <- droplevels(dat)

#subset data to get only Nov and Dec
dat<-dat[dat$Month =='Nov' | dat$Month == 'Dec',]
```

```
#create new column with 0 or 1 or represent when adult females present
dat$Present <- (dat$Sex == "female" & dat$Age == "a")
dat$Present <- as.integer(as.logical(dat$Present))

#move X20mtransect column to the end of the dataframe
dat <- dat %>%
  select(-X20mtransect, everything())

#create a new dataset where there is data for each 20m searched.

# create a new object with the different categories in it
skel <- data.frame(X20mtransect = seq(20, 100, 20))
skel$X20mtransect <- as.factor(skel$X20mtransect)

#form the new dataset by joining them together
dat1 <- dat %>%
  group_by_at(vars(Sex:Standard_time)) %>%
  nest() %>%
  mutate(data = map(data, ~full_join(.x, skel, by = "X20mtransect"))) %>%
  unnest() %>%
  replace_na(list(Present = 0))

#add time squared variable
dat1$Standard_time <- as.numeric(paste(dat1$Standard_time))
dat1$time_sq <- dat1$Standard_time*dat1$Standard_time

#make column with standardised ground temperature
dat1$Ground <- as.numeric(as.character(dat1$Ground))
dat1$Ground_standard <- (dat1$Ground - mean(dat1$Ground, na.rm = T)) /
sd(dat1$Ground, na.rm = T)
dat1$temp_sq <- dat1$Ground_standard*dat1$Ground_standard

#rename the transects so that snowy and patersons have different IDs
dat1$TransectID <- paste(dat1$Location, dat1$Transect,sep="-")

#make the new column a factor rather than a character
dat1$TransectID <- as.factor(dat1$TransectID)

#rescale season so thats its on a better scale
dat1$Season <- as.numeric(paste(dat1$Season))
dat1$SeasonID <- dat1$Season - 2015

#subset for unique rows of info
library(data.table)
dt <- data.table(dat1)
dt1<-dt[,.SD[which.max(Present)],by=list(TransectID, Visit_All_time)]

#drop rows where temp and sky contain NA
```

```
dt1 <- dt1[dt1$Ground != 'NA',]
dt1 <- dt1[dt1$Sky != 'NA',]
dt1 <- droplevels(dt1)

#check for missing values
library(Amelia)
missmap(dt1, main = "Missing values vs observed")
```




```
#make transectID include the distance too
dt1$TransectID <- paste(dt1$X20mtransect, dt1$TransectID,sep="-")

# ------------------------------
# Logistic regression on 20m data
# ------------------------------

dt1 <- droplevels(dt1)

#Look at the distribution of 0s and 1s
table(dt1$Present, dt1$Location)

##
##     Patersons Terrace Snowy River
##   0                68          75
##   1                 7          10

#run logistic regression
library(lme4)
```

```r
library(lmerTest)

#full model

#including "Season ID" in the model gives a singular fit so exluded from the
following models

#basic informative model
mod2 <- glmer(Present ~ Location + Month + Sky + (1|TransectID),  family =
"binomial", data = dt1)

mod3 <- glmer(Present ~ Location + Month + (1|TransectID),  family =
"binomial", data = dt1)

mod4 <- glmer(Present ~ Location  + (1|TransectID),  family = "binomial",
data = dt1)

anova(mod2, mod3)
```

```
## Data: dt1
## Models:
## mod3: Present ~ Location + Month + (1 | TransectID)
## mod2: Present ~ Location + Month + Sky + (1 | TransectID)
##      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## mod3  4 107.28 119.58 -49.638   99.276
## mod2  7 108.88 130.40 -47.438   94.876 4.3992      3     0.2215
```

```r
anova(mod3, mod4)
```

```
## Data: dt1
## Models:
## mod4: Present ~ Location + (1 | TransectID)
## mod3: Present ~ Location + Month + (1 | TransectID)
##      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## mod4  3 105.35 114.57 -49.674   99.348
## mod3  4 107.28 119.58 -49.638   99.276 0.0723      1     0.7881
```

```r
AIC (mod2, mod3, mod4)
```

```
##      df      AIC
## mod2  7 108.8765
## mod3  4 107.2757
## mod4  3 105.3479
```

```r
# models do not signifincalty differ, mod4 has lowest AIC

# check model fit

E1 <- residuals(mod4)
```

```
p1 <- length(fixef(mod4)) + 1
(overdisp1 <- sum(E1^2) /(nrow(dt1) - p1))

## [1] 0.3773948

summary(mod4)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: binomial  ( logit )
## Formula: Present ~ Location + (1 | TransectID)
##    Data: dt1
##
##      AIC      BIC   logLik deviance df.resid
##    105.3    114.6    -49.7     99.3      157
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.9983 -0.2189 -0.1452 -0.1294  2.8656
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  TransectID (Intercept) 5.108    2.26
## Number of obs: 160, groups:  TransectID, 35
##
## Fixed effects:
##                    Estimate Std. Error z value Pr(>|z|)
## (Intercept)         -3.3320     1.1808  -2.822  0.00477 **
## LocationSnowy River   0.7613     1.1205   0.679  0.49685
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr)
## LctnSnwyRvr -0.642

# --------------
# Plot the model
# --------------

str(dt1)

## Classes 'data.table' and 'data.frame':   160 obs. of  19 variables:
##  $ TransectID     : chr  "20-Patersons Terrace-A" "80-Patersons Terrace-C"
"40-Patersons Terrace-B" "100-Patersons Terrace-C" ...
##  $ Visit_All_time : int  6 6 6 7 7 7 8 8 8 9 ...
##  $ Sex            : Factor w/ 4 levels "0","female","male",..: 2 3 2 1 3 3
4 2 3 4 ...
##  $ Age            : Factor w/ 2 levels "a","j": 1 1 2 NA 1 1 NA 2 1 NA ...
##  $ Location       : Factor w/ 2 levels "Patersons Terrace",..: 1 1 1 1 1 1
1 1 1 1 ...
```

```
##  $ Transect       : Factor w/ 5 levels "A","B","C","D",..: 1 3 2 3 2 1 1 2
3 1 ...
##  $ Season         : num  2015 2015 2015 2015 2015 ...
##  $ Month          : Factor w/ 2 levels "Dec","Nov": 1 1 1 1 1 1 1 1 1 1
...
##  $ Ground         : num  35.1 30.7 42.7 16.2 16.2 18.2 19.8 20.9 22.6 24.6
...
##  $ Baro           : Factor w/ 75 levels "1003.5","1004.4",..: 75 72 73 64
64 65 71 69 68 71 ...
##  $ Wind           : Factor w/ 8 levels "breeze","mild",..: 4 4 4 5 7 7 2 2
3 4 ...
##  $ Sky            : Factor w/ 4 levels "high cloud","no cloud",..: 2 2 2 2
2 2 1 3 4 2 ...
##  $ Standard_time  : num  0.05 0.605 1 0.65 1.075 ...
##  $ Present        : num  1 0 0 0 0 0 0 0 0 0 ...
##  $ X20mtransect   : Factor w/ 5 levels "20","40","60",..: 1 4 2 5 5 3 1 2
2 1 ...
##  $ time_sq        : num  0.0025 0.366 1 0.4225 1.1556 ...
##  $ Ground_standard: num  1.08 0.555 1.987 -1.175 -1.175 ...
##  $ temp_sq        : num  1.166 0.308 3.947 1.381 1.381 ...
##  $ SeasonID       : num  0 0 0 0 0 0 0 0 0 0 ...
##  - attr(*, ".internal.selfref")=<externalptr>

#maketransect ID a factor
dt1$TransectID <- as.factor(dt1$TransectID)

#make transectID include the distance too
#dt1$TransectID <- paste(dt1$X20mtransect, dt1$TransectID,sep="-")

#note: make sure the order of the months is alphabetical as it is specified
in the model.
NewData <- expand.grid(Location = levels(dt1$Location),
                       SeasonID = c(0:20),
                       Month = levels(dt1$Month),
                       TransectID = levels(dt1$TransectID))

head(NewData)

##            Location SeasonID Month              TransectID
## 1 Patersons Terrace        0   Dec 100-Patersons Terrace-A
## 2       Snowy River        0   Dec 100-Patersons Terrace-A
## 3 Patersons Terrace        1   Dec 100-Patersons Terrace-A
## 4       Snowy River        1   Dec 100-Patersons Terrace-A
## 5 Patersons Terrace        2   Dec 100-Patersons Terrace-A
## 6       Snowy River        2   Dec 100-Patersons Terrace-A

X <- model.matrix(~ Location,
                  data = NewData)

NewData$eta         <- X %*% fixef(mod4)
```

```
NewData$SuccessPred <- exp(NewData$eta) / (1 + exp(NewData$eta))
head(NewData,15)
```

```
##             Location SeasonID Month              TransectID       eta
## 1  Patersons Terrace        0   Dec 100-Patersons Terrace-A -3.331955
## 2        Snowy River        0   Dec 100-Patersons Terrace-A -2.570636
## 3  Patersons Terrace        1   Dec 100-Patersons Terrace-A -3.331955
## 4        Snowy River        1   Dec 100-Patersons Terrace-A -2.570636
## 5  Patersons Terrace        2   Dec 100-Patersons Terrace-A -3.331955
## 6        Snowy River        2   Dec 100-Patersons Terrace-A -2.570636
## 7  Patersons Terrace        3   Dec 100-Patersons Terrace-A -3.331955
## 8        Snowy River        3   Dec 100-Patersons Terrace-A -2.570636
## 9  Patersons Terrace        4   Dec 100-Patersons Terrace-A -3.331955
## 10       Snowy River        4   Dec 100-Patersons Terrace-A -2.570636
## 11 Patersons Terrace        5   Dec 100-Patersons Terrace-A -3.331955
## 12       Snowy River        5   Dec 100-Patersons Terrace-A -2.570636
## 13 Patersons Terrace        6   Dec 100-Patersons Terrace-A -3.331955
## 14       Snowy River        6   Dec 100-Patersons Terrace-A -2.570636
## 15 Patersons Terrace        7   Dec 100-Patersons Terrace-A -3.331955
##    SuccessPred
## 1   0.03449106
## 2   0.07105231
## 3   0.03449106
## 4   0.07105231
## 5   0.03449106
## 6   0.07105231
## 7   0.03449106
## 8   0.07105231
## 9   0.03449106
## 10  0.07105231
## 11  0.03449106
## 12  0.07105231
## 13  0.03449106
## 14  0.07105231
## 15  0.03449106
```

```
#Get the standard errors
NewData$VarPred <- diag(X %*% vcov(mod4) %*% t(X))
NewData$sePred  <- sqrt(NewData$VarPred)

NewData$seLow  <- exp(NewData$eta - 1.96 * NewData$sePred) / (1 +
exp(NewData$eta - 1.96 * NewData$sePred))
NewData$seHigh <- exp(NewData$eta + 1.96 * NewData$sePred) / (1 +
exp(NewData$eta + 1.96 * NewData$sePred))
head(NewData, 10)
```

```
##             Location SeasonID Month              TransectID       eta
## 1  Patersons Terrace        0   Dec 100-Patersons Terrace-A -3.331955
## 2        Snowy River        0   Dec 100-Patersons Terrace-A -2.570636
## 3  Patersons Terrace        1   Dec 100-Patersons Terrace-A -3.331955
```

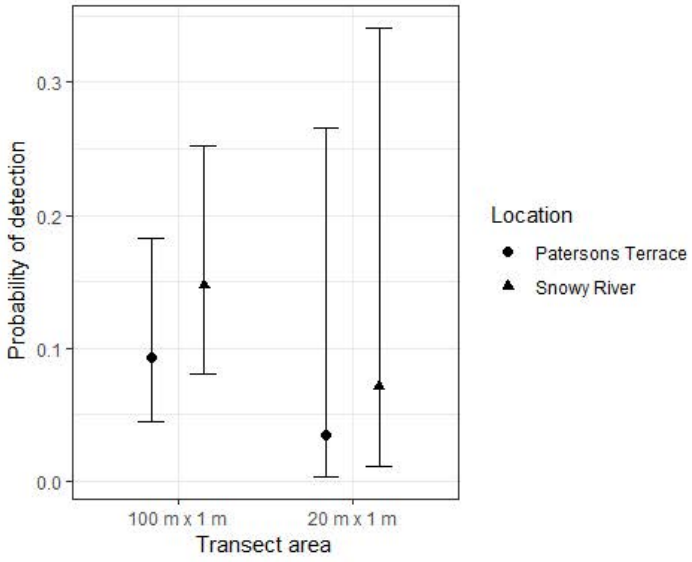
```
## 4        Snowy River        1   Dec 100-Patersons Terrace-A -2.570636
## 5  Patersons Terrace        2   Dec 100-Patersons Terrace-A -3.331955
## 6        Snowy River        2   Dec 100-Patersons Terrace-A -2.570636
## 7  Patersons Terrace        3   Dec 100-Patersons Terrace-A -3.331955
## 8        Snowy River        3   Dec 100-Patersons Terrace-A -2.570636
## 9  Patersons Terrace        4   Dec 100-Patersons Terrace-A -3.331955
## 10       Snowy River        4   Dec 100-Patersons Terrace-A -2.570636
##    SuccessPred   VarPred    sePred       seLow    seHigh
## 1   0.03449106 1.3942237 1.1807725 0.003518242 0.2654871
## 2   0.07105231 0.9504138 0.9748917 0.011190641 0.3407737
## 3   0.03449106 1.3942237 1.1807725 0.003518242 0.2654871
## 4   0.07105231 0.9504138 0.9748917 0.011190641 0.3407737
## 5   0.03449106 1.3942237 1.1807725 0.003518242 0.2654871
## 6   0.07105231 0.9504138 0.9748917 0.011190641 0.3407737
## 7   0.03449106 1.3942237 1.1807725 0.003518242 0.2654871
## 8   0.07105231 0.9504138 0.9748917 0.011190641 0.3407737
## 9   0.03449106 1.3942237 1.1807725 0.003518242 0.2654871
## 10  0.07105231 0.9504138 0.9748917 0.011190641 0.3407737
```

```
#reorder the levels so that they plto correctly
NewData$Month2 <- factor(NewData$Month, levels = c("Nov", "Dec", "Jan",
"Feb", "Mar"))

dat20 <- data.frame(NewData, stringsAsFactors=F)

# ----------------------------------
# Graph the findings for adult females
# ----------------------------------

#join dat20 and dat100 together

dat20$Area <- c("20 m x 1 m")
dat100$Area <- c("100 m x 1 m")

dat20 <- data.frame(dat20, stringsAsFactors=F)
dat100 <- data.frame(dat100, stringsAsFactors=F)

#drop the transect ID columns
dat20$TransectID <- NULL
dat20$Area <- as.factor(dat20$Area)
dat20 <- droplevels(dat20)

str(dat20)
```

```
## 'data.frame':    2940 obs. of  11 variables:
##  $ Location   : Factor w/ 2 levels "Patersons Terrace",..: 1 2 1 2 1 2 1 2
1 2 ...
##  $ SeasonID   : int  0 0 1 1 2 2 3 3 4 4 ...
##  $ Month      : Factor w/ 2 levels "Dec","Nov": 1 1 1 1 1 1 1 1 1 1 ...
##  $ eta        : num [1:2940, 1] -3.33 -2.57 -3.33 -2.57 -3.33 ...
```

```
##   ..- attr(*, "dimnames")=List of 2
##   .. ..$ : chr  "1" "2" "3" "4" ...
##   .. ..$ : NULL
##  $ SuccessPred: num [1:2940, 1] 0.0345 0.0711 0.0345 0.0711 0.0345 ...
##   ..- attr(*, "dimnames")=List of 2
##   .. ..$ : chr  "1" "2" "3" "4" ...
##   .. ..$ : NULL
##  $ VarPred    : num  1.39 0.95 1.39 0.95 1.39 ...
##  $ sePred     : num  1.181 0.975 1.181 0.975 1.181 ...
##  $ seLow      : num [1:2940, 1] 0.00352 0.01119 0.00352 0.01119 0.00352
...
##   ..- attr(*, "dimnames")=List of 2
##   .. ..$ : chr  "1" "2" "3" "4" ...
##   .. ..$ : NULL
##  $ seHigh     : num [1:2940, 1] 0.265 0.341 0.265 0.341 0.265 ...
##   ..- attr(*, "dimnames")=List of 2
##   .. ..$ : chr  "1" "2" "3" "4" ...
##   .. ..$ : NULL
##  $ Month2     : Factor w/ 2 levels "Nov","Dec": 2 2 2 2 2 2 2 2 2 2 ...
##  $ Area       : Factor w/ 1 level "20 m x 1 m": 1 1 1 1 1 1 1 1 1 1 ...
```

```
dat100$TransectID <- NULL
dat100$Area <- as.factor(dat100$Area)
dat100 <- droplevels(dat100)

dat.all <- rbind(do.call(data.frame,dat100), do.call(data.frame, dat20))

#plot the graph ----

dodge <- position_dodge(width=0.5)

library(ggplot2)


ggplot(data = dat.all, aes(Area, SuccessPred, shape=Location)) +
  geom_point(aes(shape=Location), size = 2, position=position_dodge(width =
0.6)) + #plot the points
  geom_errorbar(aes(ymin=seLow, ymax=seHigh), width = 0.3,
position=position_dodge(width=0.6)) +
 # facet_wrap(~Area) +
  theme(axis.text.x = element_text(face = "plain", color = "black",
                                   size = 12)) +
  theme_bw() +
  labs(x = "Transect area", y = (expression("Probability of detection")))
```


Probability of detection
0.3
0.2
0.1
0.0
100 m x 1 m
20 m x 1 m
Transect area
Location
Patersons Terrace
Snowy River

Appendix I

Appendix B: Analyses and R code

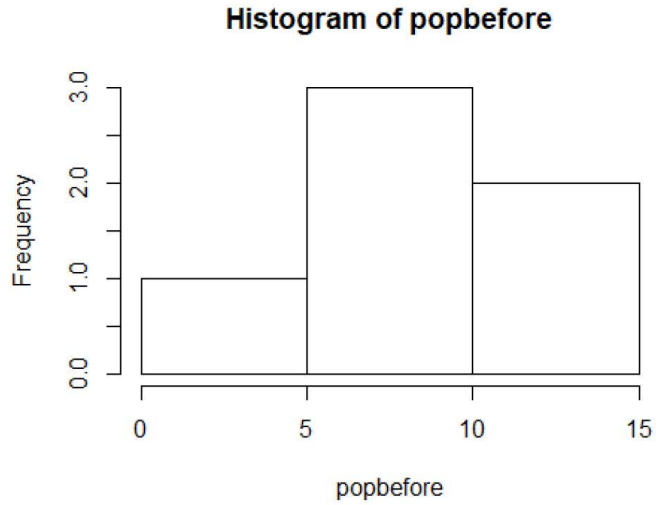
Appendix B : R code and analyses

Bootstrap setup

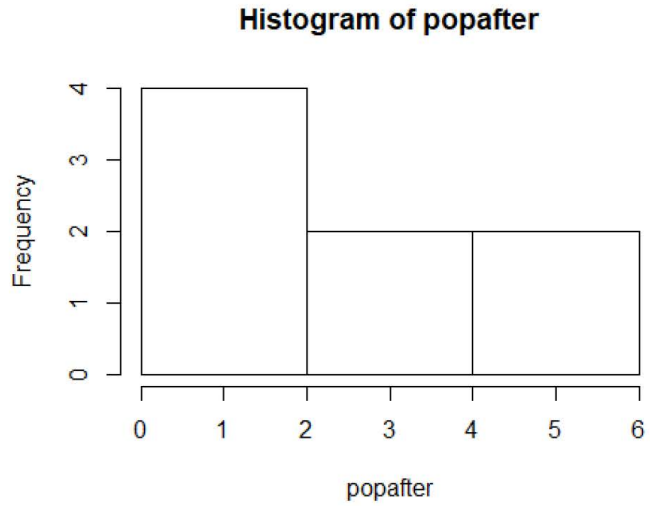
```
# -------------------------
# bootstrapping preparation ----
# -------------------------

set.seed(123456)
B <- 10000 #number of boostraps
Boot.test.stat1 <- rep(0,B)
```

Population Size: Before and After Flood

```
# ---------------------------------------
# Population Size: Before and After Flood ----
# ---------------------------------------

# data
popbefore <- c(8,3,15,10,14,10)
popafter <- c(3,6,5,4,0,2,1,2)

print(mean(popbefore))

## [1] 10

print(mean(popafter))

## [1] 2.875

#check assumptions - very small sample size
var.test(popbefore, popafter)

##
##  F test to compare two variances
##
## data:  popbefore and popafter
## F = 4.5576, num df = 5, denom df = 7, p-value = 0.07221
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##   0.862322 31.233411
## sample estimates:
## ratio of variances
##           4.557576

hist(popbefore)
```




```
hist(popafter)
```



```
#data in long format
popn <- as.factor(rep(c("popbefore", "popafter"), times = c(6,8)))
count <- c(8,3,15,10,14,10,3,6,5,4,0,2,1,2)

#reshape
dat1 <- data.frame(popn, count)

# factors of bootstrap test
n <- length(dat1$popn)
variable <- dat1$count
BootstrapSamples <- matrix(sample(variable, size = n*B, replace = T), nrow=n,
ncol=B)

# run bootstrap test
for (i in 1:B){
  Boot.test.stat1[i] <- abs(mean(BootstrapSamples[1:6,i]) -
mean(BootstrapSamples[7:14,i]))
    }

# test statistic
test.stat1 <- abs(mean(dat1$count[dat1$popn=="popbefore"]) -
mean(dat1$count[dat1$popn=="popafter"]))

#print
test.stat1
```

```
## [1] 7.125
```

```
#p-valaue for means
pval <- mean(Boot.test.stat1 >= test.stat1)

#print
pval # sig diff
```

```
## [1] 0.0033
```

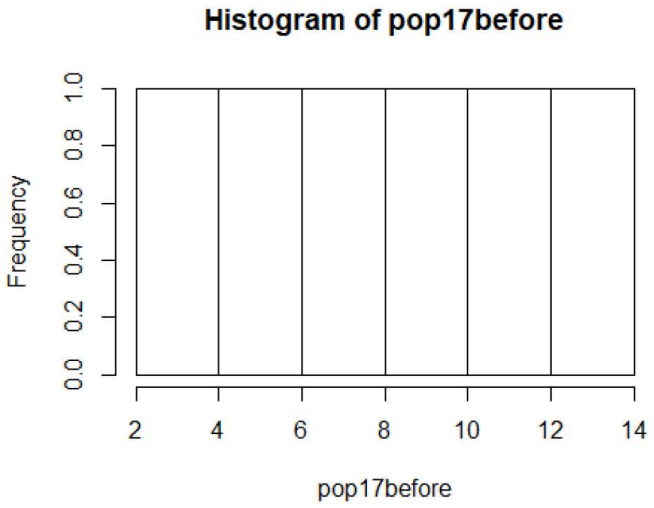
Population size: Before and After Year 2016-17

```
# ------------------------------------------------
# Population size: Before and After Year 2016-17 ----
# ------------------------------------------------

# read in data
pop17before <- c(8,10,3,13,11,6)
pop17after <- c(6,13,11,10,16)

print(mean(pop17before))
```

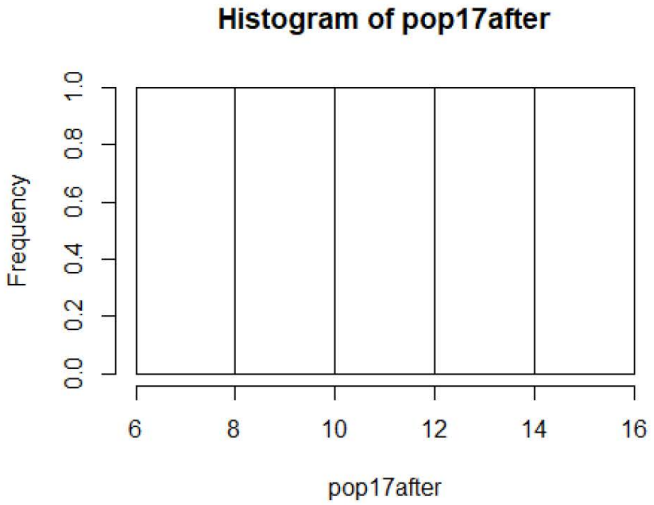
```
## [1] 8.5
```

```
print(mean(pop17after))
```

```
## [1] 11.2

# check assumptions - not normal distributions, small sample sizes
var.test(pop17before, pop17after)

##
##  F test to compare two variances
##
## data:  pop17before and pop17after
## F = 0.9562, num df = 5, denom df = 4, p-value = 0.9357
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.1021098 7.0643287
## sample estimates:
## ratio of variances
##          0.9562044

hist(pop17before)
```




```
hist(pop17after)
```

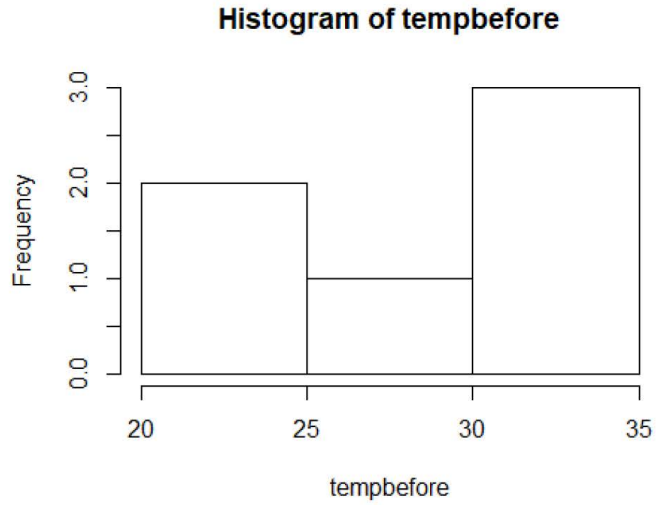



```
#data in long format
popn <- as.factor(rep(c("pop17before", "pop17after"), times = c(6,5)))
count <- c(8,10,3,13,11,6,6,13,11,10,16)

#reshape
dat2 <- data.frame(popn, count)

# look at data
table(dat2$popn)

##
##  pop17after pop17before
##           5           6

# factors of bootstrap test
n <- length(dat2$popn)
variable <- dat2$count
BootstrapSamples <- matrix(sample(variable, size = n*B, replace = T), nrow=n,
ncol=B)

# run bootstrap test
for (i in 1:B){
  Boot.test.stat1[i] <- abs(mean(BootstrapSamples[1:5,i]) -
mean(BootstrapSamples[6:11,i]))
}

# test statistic
```

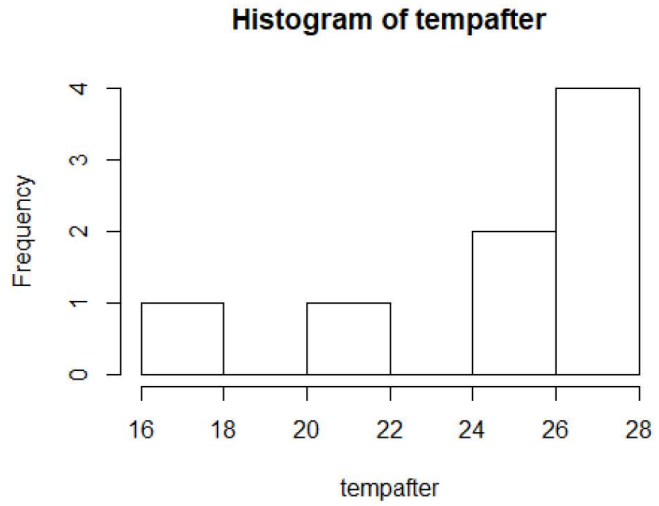
```
test.stat1 <- abs(mean(dat2$count[dat2$popn=="pop17before"]) -
mean(dat2$count[dat2$popn=="pop17after"]))

#print
test.stat1

## [1] 2.7

#p-valaue for means
pval1 <- mean(Boot.test.stat1 >= test.stat1)

#print
pval1 #non-sig diff

## [1] 0.2198
```

Temperature: Before and After Flood

```
# ----------------------------------
# Temperature: Before and After Flood ----
# ----------------------------------

# read in data
tempbefore <- c(33.3, 26.7, 34.2, 24.7, 23, 30.4)
tempafter <- c(25.9, 26.8, 17.7, 24.5, 21.7, 27.8, 26.1, 26.8)

print(mean(tempbefore))

## [1] 28.71667

print(mean(tempafter))

## [1] 24.6625

#check assumptions - non normal data, and small sample size
var.test(tempbefore,tempafter)

##
##  F test to compare two variances
##
## data:  tempbefore and tempafter
## F = 1.8721, num df = 5, denom df = 7, p-value = 0.4355
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##   0.3542133 12.8296515
## sample estimates:
## ratio of variances
##           1.872101

hist(tempbefore)
```

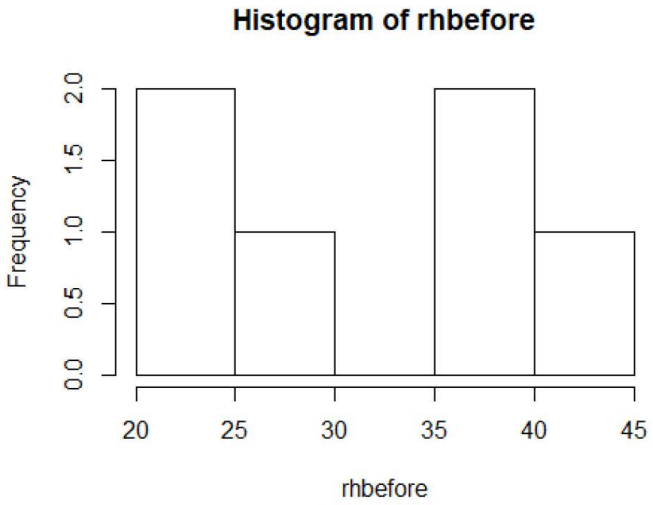



```
hist(tempafter)
```

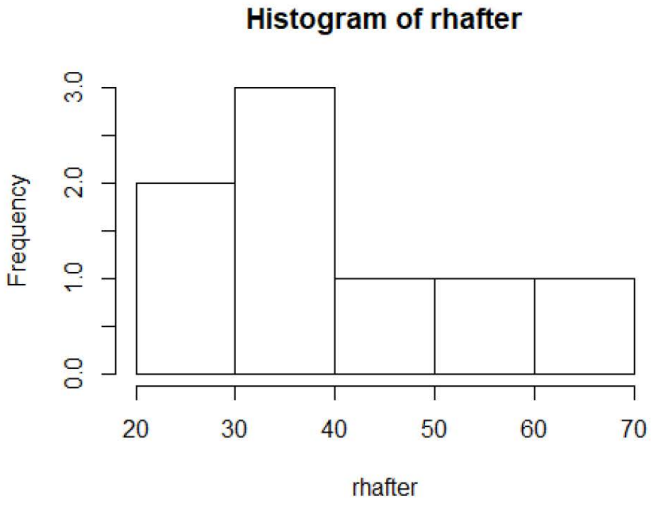


```r
#data in long format
when <- as.factor(rep(c("tempbefore", "tempafter"), times = c(6,8)))
temp <- c(33.3, 26.7, 34.2, 24.7, 23, 30.4, 25.9, 26.8, 17.7, 24.5, 21.7,
27.8, 26.1, 26.8)

#reshape
dat3 <- data.frame(when, temp)

# look at data
table(dat3$when)

##
##  tempafter tempbefore
##          8          6

# factors of bootstrap test
n <- length(dat3$when)
variable <- dat3$temp
BootstrapSamples <- matrix(sample(variable, size = n*B, replace = T), nrow=n,
ncol=B)

# run bootstrap test
for (i in 1:B){
  Boot.test.stat1[i] <- abs(mean(BootstrapSamples[1:6,i]) -
mean(BootstrapSamples[7:14,i]))
}

# test statistic
test.stat1 <- abs(mean(dat3$temp[dat3$when=="tempbefore"]) -
mean(dat3$temp[dat3$when=="tempafter"]))

#print
test.stat1

## [1] 4.054167

#p-valaue for means
pval2 <- mean(Boot.test.stat1 >= test.stat1)

#print
pval2 #non-sig diff

## [1] 0.0721

# rh: Before and After ----

rhbefore <- c(27.8, 36.3, 22.5, 39.5, 44.5, 22.7)
rhafter <- c(50.5, 45.7, 68, 37.2, 34.7, 21.9, 29.4, 38.3)

print(mean(rhbefore))
```

```
## [1] 32.21667

print(mean(rhafter))

## [1] 40.7125

#check assumptions - non normal data, and small sample size
var.test(rhbefore,rhafter)

##
##  F test to compare two variances
##
## data:  rhbefore and rhafter
## F = 0.42464, num df = 5, denom df = 7, p-value = 0.3628
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.08034509 2.91010948
## sample estimates:
## ratio of variances
##          0.4246428

hist(rhbefore)
```




```
hist(rhafter)
```




```
#data in long format
when <- as.factor(rep(c("rhbefore", "rhafter"), times = c(6,8)))
rh <- c(27.8, 36.3, 22.5, 39.5, 44.5, 22.7, 50.5, 45.7, 68, 37.2, 34.7, 21.9,
29.4, 38.3)

#reshape
dat3 <- data.frame(when, rh)

# look at data
table(dat3$when)

##
##  rhafter rhbefore
##        8        6

# factors of bootstrap test
n <- length(dat3$when)
variable <- dat3$rh
BootstrapSamples <- matrix(sample(variable, size = n*B, replace = T), nrow=n,
ncol=B)

# run bootstrap test
for (i in 1:B){
  Boot.test.stat1[i] <- abs(mean(BootstrapSamples[1:6,i]) -
mean(BootstrapSamples[7:14,i]))
}
```

```
# test statistic
test.stat1 <- abs(mean(dat3$rh[dat3$when=="rhbefore"]) -
mean(dat3$rh[dat3$when=="rhafter"]))

#print
test.stat1
```

```
## [1] 8.495833
```

```
#p-valaue for means
pval2 <- mean(Boot.test.stat1 >= test.stat1)

#print
pval2 #non-sig diff
```

```
## [1] 0.1931
```

Body length: Before and After

```
# ----------------------------
# Body length: Before and After ----
# ----------------------------

## FEMALES ----


fbefore <-
c(14,14,19,14,15,15,15,13,13,14,14,16,14,12,23,15,13,19,14,8,14,20,15,16,18,1
9,18,17)
fafter <- c(14,16,15,16,16,18,17,18,16,15,15,16,21,15,23,21,17,21,17,23,28)

print(mean(fbefore))
```

```
## [1] 15.39286
```

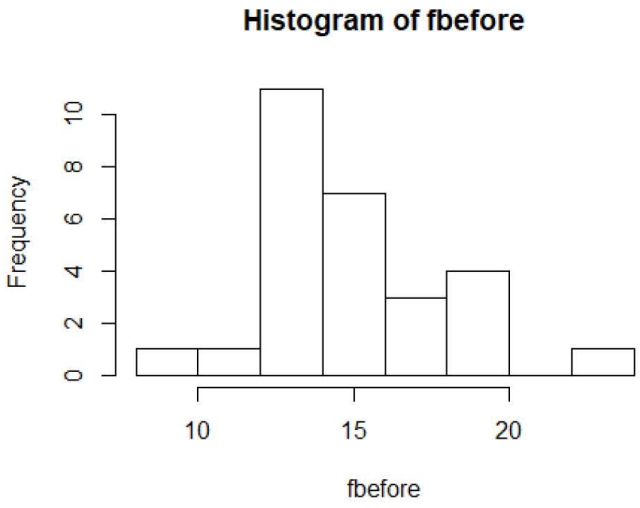
```
print(mean(fafter))
```

```
## [1] 18
```

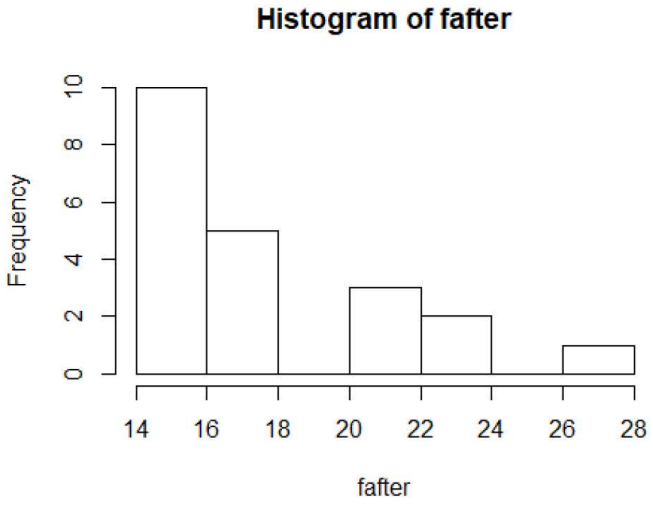
```
#test assumptions - not normal, small sample sizes
var.test(fbefore,fafter)
```

```
##
##  F test to compare two variances
##
## data:  fbefore and fafter
## F = 0.68983, num df = 27, denom df = 20, p-value = 0.3644
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.2904436 1.5543655
## sample estimates:
```

```
## ratio of variances
##          0.6898253

hist(fbefore)
```




```
hist(fafter)
```




```
#data in long format
when <- as.factor(rep(c("fbefore", "fafter"), times = c(28,21)))
length <-
c(14,14,19,14,15,15,15,13,13,14,14,16,14,12,23,15,13,19,14,8,14,20,15,16,18,1
9,18,17,14,16,15,16,16,18,17,18,16,15,15,16,21,15,23,21,17,21,17,23,28)

#reshape
dat4 <- data.frame(when, length)

# look at data
table(dat4$when)

##
##  fafter fbefore
##      21      28

# factors of bootstrap test
n <- length(dat4$when)
variable <- dat4$length
BootstrapSamples <- matrix(sample(variable, size = n*B, replace = T), nrow=n,
ncol=B)

# run bootstrap test
for (i in 1:B){
  Boot.test.stat1[i] <- abs(mean(BootstrapSamples[1:28,i]) -
mean(BootstrapSamples[29:49,i]))
}
```

```r
# test statistic
test.stat1 <- abs(mean(dat4$length[dat4$when=="fbefore"]) -
mean(dat4$length[dat4$when=="fafter"]))

#print
test.stat1
```

```
## [1] 2.607143
```

```r
#p-valaue for means
pval3 <- mean(Boot.test.stat1 >= test.stat1)

#print
pval3 #non-sig diff
```

```
## [1] 0.0077
```

```r
## MALES ----


mbefore <-
c(16,17,12.5,19,13,11,11,17,14,12,18,18,14,17,19,12,14,15,13,19,18,16)
mafter <- c(16,13)

print(mean(mbefore))
```

```
## [1] 15.25
```

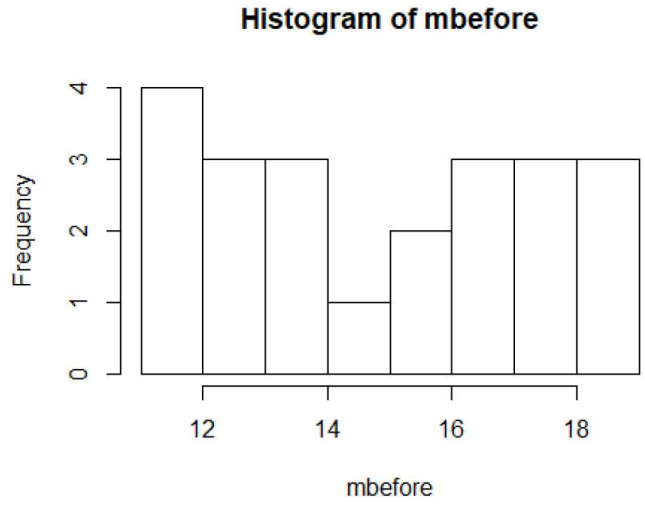
```r
print(mean(mafter))
```

```
## [1] 14.5
```

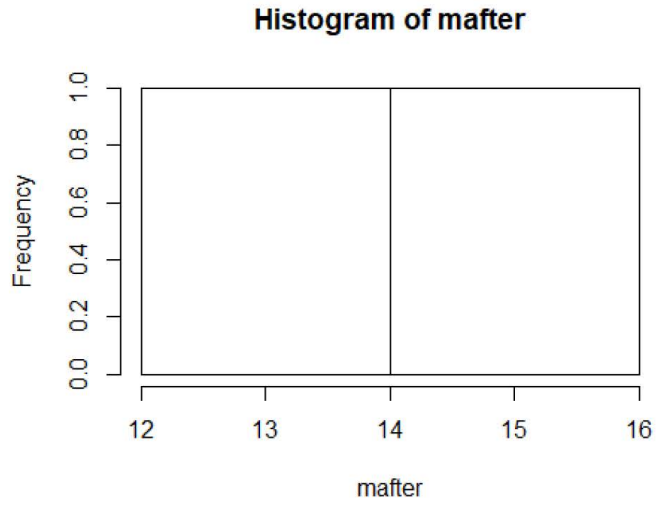
```r
# test assumptions - not normal, small and uneven sample sizes
var.test(mbefore,mafter)
```

```
##
##  F test to compare two variances
##
## data:  mbefore and mafter
## F = 1.6389, num df = 21, denom df = 1, p-value = 0.8869
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.001648308 9.549228280
## sample estimates:
## ratio of variances
##           1.638889
```

```r
hist(mbefore)
```


Histogram of mbefore
Frequency
0
1
2
3
4
12
14
16
18
mbefore

```
hist(mafter)
```

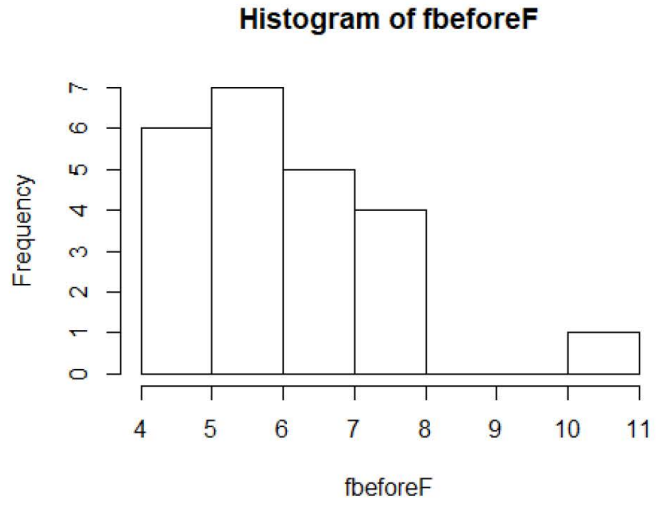

Histogram of mafter
Frequency
0.0
0.2
0.4
0.6
0.8
1.0
12
13
14
15
16
mafter

```
#data in long format
when <- as.factor(rep(c("mbefore", "mafter"), times = c(22,2)))
length <-
c(16,17,12.5,19,13,11,11,17,14,12,18,18,14,17,19,12,14,15,13,19,18,16,16,13)

#reshape
dat5 <- data.frame(when, length)

# look at data
table(dat5$when)

##
##  mafter mbefore
##       2      22

# factors of bootstrap test
n <- length(dat5$when)
variable <- dat5$length
BootstrapSamples <- matrix(sample(variable, size = n*B, replace = T), nrow=n,
ncol=B)

# run bootstrap test
for (i in 1:B){
  Boot.test.stat1[i] <- abs(mean(BootstrapSamples[1:22,i]) -
mean(BootstrapSamples[23:24,i]))
}

# test statistic
test.stat1 <- abs(mean(dat5$length[dat5$when=="mbefore"]) -
mean(dat5$length[dat5$when=="mafter"]))

#print
test.stat1

## [1] 0.75

#p-valaue for means
pval4 <- mean(Boot.test.stat1 >= test.stat1)

#print
pval4#non-sig diff

## [1] 0.7103
```

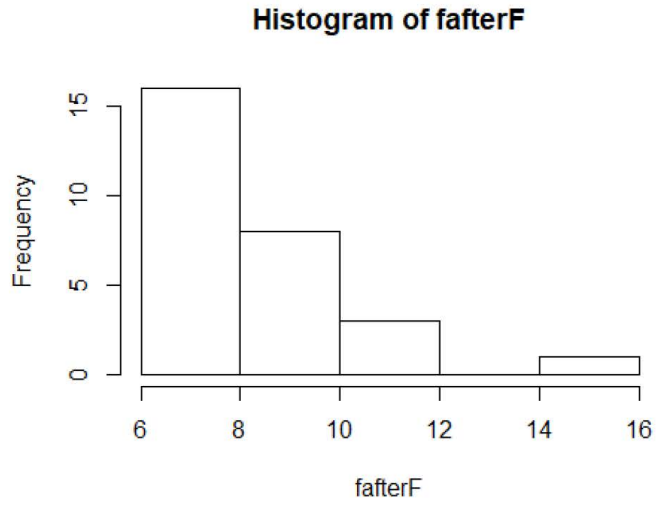
Femur length: female before and after

```
# -------------------------------------
# Femur length: female before and after ----
# -------------------------------------

# 2017 female ----
```

```
fbeforeF <- c(5, 5, 6.5, 5, 6.5, 5.5, 5, 6, 4.5, 7, 8, 6, 7, 6, 5.5, 7, 6, 6,
5, 8, 7.5, 11, 7.5)
fafterF <-
c(8,11.5,7,7.4,11.4,7.6,7.3,7.7,7.1,9,8.5,7,10,7.5,7,10.5,8,9,7.5,7,7,7.5,9.5
,9,9,6,16,10)

print(mean(fbeforeF))

## [1] 6.369565

print(mean(fafterF))

## [1] 8.571429

#assumption testing - unequal variance, small sample sizes, non normal
distributions
var.test(fbeforeF,fafterF) # sig diff variances

##
##  F test to compare two variances
##
## data:  fbeforeF and fafterF
## F = 0.50392, num df = 22, denom df = 27, p-value = 0.1048
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.2268335 1.1583386
## sample estimates:
## ratio of variances
##          0.5039228

hist(fbeforeF)
```




```
hist(fafterF)
```



```r
#data in long format
when <- as.factor(rep(c("fbeforeF", "fafterF"), times = c(23,28)))
length <-
c(5,5,6.5,5,6.5,5.5,5,6,4.5,7,8,6,7,6,5.5,7,6,6,5,8,7.5,11,7.5,8,11.5,7,7.4,1
1.4,7.6,7.3,7.7,7.1,9,8.5,7,10,7.5,7,10.5,8,9,7.5,7,7,7.5,9.5,9,9,6,16,10)

#reshape
dat6 <- data.frame(when, length)

# look at data
table(dat6$when)

##
##  fafterF fbeforeF
##       28       23

# factors of bootstrap test
n <- length(dat6$when)
variable <- dat6$length
BootstrapSamples <- matrix(sample(variable, size = n*B, replace = T), nrow=n,
ncol=B)

# run bootstrap test
for (i in 1:B){
  Boot.test.stat1[i] <- abs(mean(BootstrapSamples[1:23,i]) -
mean(BootstrapSamples[24:51,i]))
}

# test statistic
test.stat1 <- abs(mean(dat6$length[dat6$when=="fbeforeF"]) -
mean(dat6$length[dat6$when=="fafterF"]))

#print
test.stat1

## [1] 2.201863

#p-valaue for means
pval5 <- mean(Boot.test.stat1 >= test.stat1)

#print
pval5 #sig diff

## [1] 3e-04

# 2018 femlale ----


fbefore18 <- c(6,8,8,6,6,8,6,8,8,8,6,8,8,8,14,8,8,10,10,6.5,8,10,8,7,8,8,8,9)
fafter18 <- c(7,10,7,9,7,9,11,12,10,10,9,11,12,7,9,9,10,9,9,12,17)
```

```
print(mean(fbefore18))
```

```
## [1] 8.017857
```

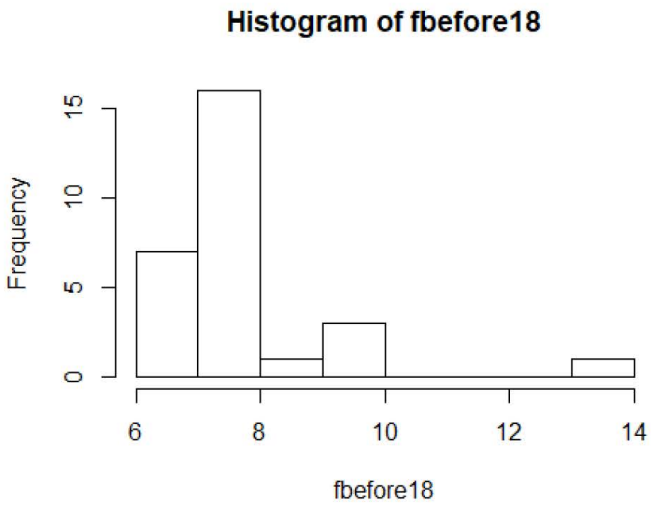
```
print(mean(fafter18))
```

```
## [1] 9.809524
```

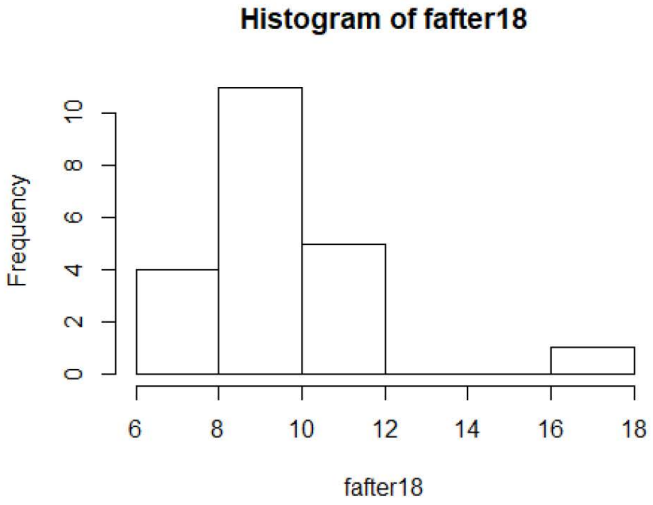
```
#check assumptions - small sample size, not normal distribution
var.test(fbefore18, fafter18)
```

```
##
##  F test to compare two variances
##
## data:  fbefore18 and fafter18
## F = 0.50848, num df = 27, denom df = 20, p-value = 0.1017
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.2140919 1.1457542
## sample estimates:
## ratio of variances
##          0.5084842
```

```
hist(fbefore18)
```




```
hist(fafter18)
```




```
#data in long format
when <- as.factor(rep(c("fbefore18", "fafter18"), times = c(28,21)))
length <-
c(6,8,8,6,6,8,6,8,8,8,6,8,8,8,14,8,8,10,10,6.5,8,10,8,7,8,8,8,9,7,10,7,9,7,9,
11,12,10,10,9,11,12,7,9,9,10,9,9,12,17)

#reshape
dat7 <- data.frame(when, length)

# look at data
table(dat7$when)

##
##  fafter18 fbefore18
##        21        28

# factors of bootstrap test
n <- length(dat7$when)
variable <- dat7$length
BootstrapSamples <- matrix(sample(variable, size = n*B, replace = T), nrow=n,
ncol=B)

# run bootstrap test
for (i in 1:B){
  Boot.test.stat1[i] <- abs(mean(BootstrapSamples[1:28,i]) -
mean(BootstrapSamples[29:49,i]))
}
```

```
# test statistic
test.stat1 <- abs(mean(dat7$length[dat7$when=="fbefore18"]) -
mean(dat7$length[dat7$when=="fafter18"]))

#print
test.stat1
```

```
## [1] 1.791667
```

```
#p-valaue for means
pval6 <- mean(Boot.test.stat1 >= test.stat1)

#print
pval6 #sig diff
```

```
## [1] 0.0038
```

Femur length: male before and after

```
# -------------------------------------
# Femur length: male before and after ----
# -------------------------------------

# 2017 male ----

mbeforeF <- c(5,5,6,6,5,7,6,5.5,6,5.5,6,7,6.5,7.5,5,7,7,8,5,7,5.5,6,5,6,7)
mafterF <- c(6,6,7,9.6,8.3,6.7,6,8.7,8.4,9,9,9,7,9,5.5,9,7.5,9.5,9,9,7.5,9,9)

print(mean(mbeforeF))
```

```
## [1] 6.1
```

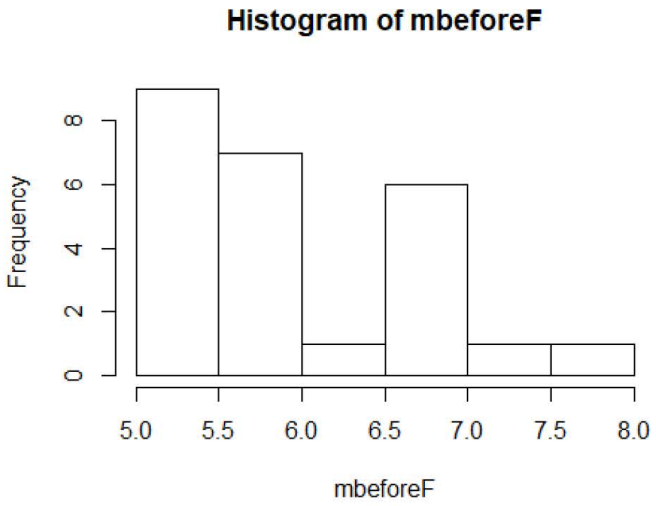
```
print(mean(mafterF))
```

```
## [1] 8.030435
```

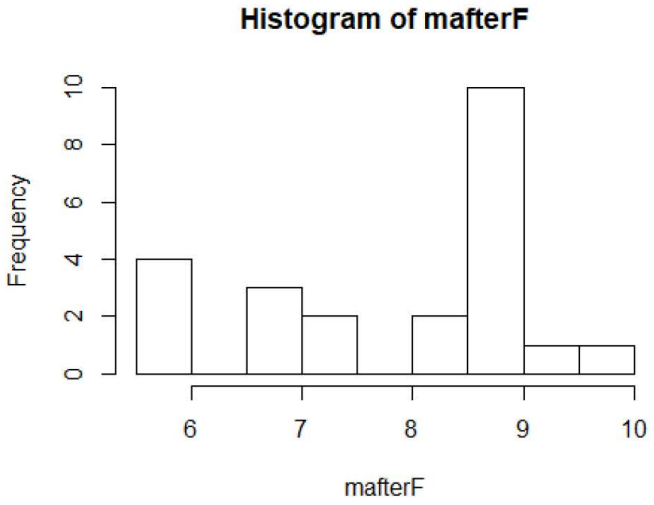
```
#check assumptions - sig diff variance, not normal distribution, small sample
size
var.test(mbeforeF, mafterF) #sig different
```

```
##
##  F test to compare two variances
##
## data:  mbeforeF and mafterF
## F = 0.47112, num df = 24, denom df = 22, p-value = 0.07527
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##  0.2020671 1.0816454
## sample estimates:
```

```
## ratio of variances
##          0.4711193

hist(mbeforeF)
```




```
hist(mafterF)
```




```
#data in long format
when <- as.factor(rep(c("mbeforeF", "mafterF"), times = c(25,23)))
length <-
c(5,5,6,6,5,7,6,5.5,6,5.5,6,7,6.5,7.5,5,7,7,8,5,7,5.5,6,5,6,7,6,6,7,9.6,8.3,6
.7,6,8.7,8.4,9,9,9,7,9,5.5,9,7.5,9.5,9,9,7.5,9,9)

#reshape
dat8 <- data.frame(when, length)

# look at data
table(dat8$when)

##
##  mafterF mbeforeF
##       23       25

# factors of bootstrap test
n <- length(dat8$when)
variable <- dat8$length
BootstrapSamples <- matrix(sample(variable, size = n*B, replace = T), nrow=n,
ncol=B)

# run bootstrap test
for (i in 1:B){
  Boot.test.stat1[i] <- abs(mean(BootstrapSamples[1:25,i]) -
mean(BootstrapSamples[26:48,i]))
}
```

```r
# test statistic
test.stat1 <- abs(mean(dat8$length[dat8$when=="mbeforeF"]) -
mean(dat8$length[dat8$when=="mafterF"]))

#print
test.stat1
```

```
## [1] 1.930435
```

```r
#p-valaue for means
pval7 <- mean(Boot.test.stat1 >= test.stat1)

#print
pval7 #sig diff
```

```
## [1] 0
```

```r
# 2018 male ----


mbefore18 <- c(8,9,7,7.5,6,5.5,7,7,7,5.5,9,6,6,10,7,6,7,6,8,8,7,7)
mafter18 <- c(8,7)

print(mean(mbefore18))
```

```
## [1] 7.113636
```

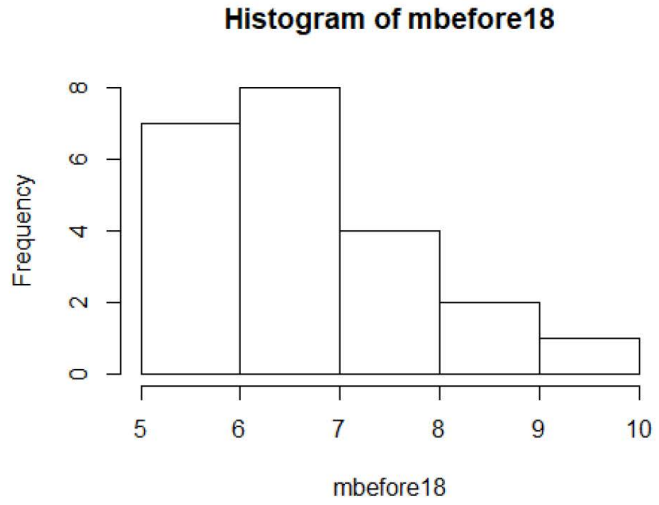
```r
print(mean(mafter18))
```

```
## [1] 7.5
```

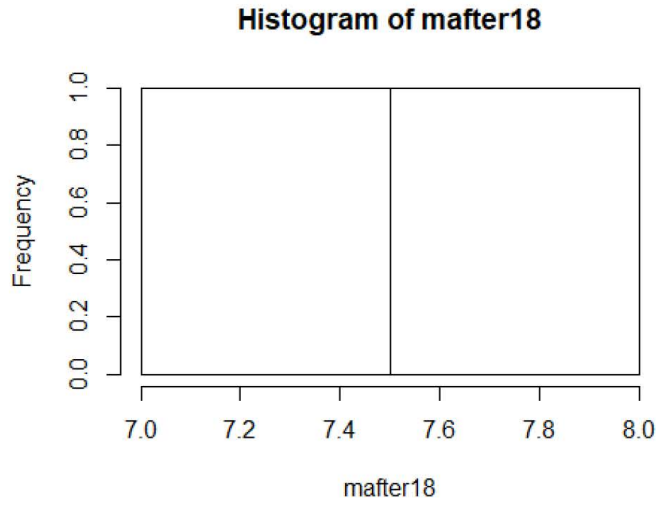
```r
#check assumptions - not normal distribution, small sample size
var.test(mbefore18, mafter18)
```

```
##
##  F test to compare two variances
##
## data:  mbefore18 and mafter18
## F = 2.8063, num df = 21, denom df = 1, p-value = 0.8861
## alternative hypothesis: true ratio of variances is not equal to 1
## 95 percent confidence interval:
##   0.002822405 16.351187936
## sample estimates:
## ratio of variances
##           2.806277
```

```r
hist(mbefore18)
```




```
hist(mafter18)
```



```r
#data in long format
when <- as.factor(rep(c("mbefore18", "mafter18"), times = c(22,2)))
length <- c(8,9,7,7.5,6,5.5,7,7,7,5.5,9,6,6,10,7,6,7,6,8,8,7,7,8,7)

#reshape
dat9 <- data.frame(when, length)

# look at data
table(dat9$when)

##
##  mafter18 mbefore18
##         2        22

# factors of bootstrap test
n <- length(dat9$when)
variable <- dat9$length
BootstrapSamples <- matrix(sample(variable, size = n*B, replace = T), nrow=n,
ncol=B)

# run bootstrap test
for (i in 1:B){
  Boot.test.stat1[i] <- abs(mean(BootstrapSamples[1:22,i]) -
mean(BootstrapSamples[23:24,i]))
}

# test statistic
test.stat1 <- abs(mean(dat9$length[dat9$when=="mbefore18"]) -
mean(dat9$length[dat9$when=="mafter18"]))

#print
test.stat1

## [1] 0.3863636

#p-valaue for means
pval8 <- mean(Boot.test.stat1 >= test.stat1)

#print
pval8 #non-sig diff

## [1] 0.6574
```